Investigating Factors Affecting the Anaerobic Digestion of Seaweed: Modelling and Experimental Approaches –

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Investigating factors affecting the anaerobic digestion of seaweed: modelling and experimental approaches

by

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I certify that this is a true and accurate version of the thesis approved by the examiners.

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Anthony Hierholtzer

À ma famille...

Abstract

The use of alternative feedstock sources to enhance the energy production of anaerobic systems, and thus their economic value, is one of the current research areas in the field of bioenergy production. Marine biomass represents a unique source of organic matter for the optimisation of anaerobic digestion systems and can be regarded as a sustainable alternative to purposely grown energy crops requiring significant amounts of water, fertiliser and land for their cultivation. Seaweeds are of particular interest as they are characterised by high biomass yields and interesting conversion rates. In temperate seas, brown seaweed species generally dominate the flora and their relative abundance on the sublittoral zone of the British coastline make them a substrate of choice for anaerobic digestion. However, little information is available on commercial-scale anaerobic digestion of seaweed for biogas production and the potential factors that could impair its successful conversion. This work was proposed in order to establish the potential and optimise the use of seaweed as an additional source of organic matter for anaerobic digesters. The study also investigated the use of the Anaerobic Digestion Model No.1 (ADM1) as a platform for process simulation. The model original structure is inadequate to accurately represent the anaerobic co-digestion of seaweed and was therefore updated with the addition of specific processes. The study was carried out in three main experimental stages. In a first stage, the effect of seaweed salinity (represented by sodium ions) on anaerobic digestion was investigated using a mesophilic laboratory-scale anaerobic digester. It was found that a rapid increase in sodium ion levels can negatively impact on biogas production and result in the accumulation of volatile fatty acids. The ADM1 does not originally take into account the inhibitory effect of sodium and was therefore modified to include a function representing the effect of sodium ions on the rate of acetate uptake. The extended model was able to reproduce experimental observations and was used to predict the effect of sodium ions in the presence of other process inhibitors. Microbial adaptation to salinity was also investigated during batch assays. It was found that a suitable period of adaptation can significantly reduce the adverse effect of salinity on methanogens. The phenomenon was successfully implemented in the model through the addition of a specific inhibition function and the calibration of kinetic parameters. The second stage of this research focused on the effect and mode of action of phlorotannin (a phenolic compound found exclusively in brown seaweed) on mixed microbial cultures through the monitoring of intra-cellular material leakage and transmission electron microscopy observations. Results suggested that phlorotannin induces strong extra- and intra-cellular effects on cells exposed to the compound, thus adversely impacting on energy requirements and final methane yields. The effect of phlorotannin was found to be dependent on both the degree of polymerisation of the compound and the morphology of microorganisms. Furthermore, the effect of phlorotannin during the anaerobic co-digestion of brown seaweed (*Laminaria digitata*) and vegetable residues was also investigated. Experimental results were successfully modelled using an extensively modified version of the ADM1, which introduces an uncompetitive function to the rate of acetate uptake in order to represent the inhibition of methanogenesis by phlorotannin. The model was also updated with a combination module for the simulation of co-digestion processes. The third stage focused on establishing operational guidelines for the anaerobic co-digestion of brown seaweed and non-saline feedstocks. Results suggested that although seaweed can be an alternative organic substrate in anaerobic digestion systems, phlorotannin content might limit its use for commercial-scale application. Whilst this study identified salinity and phlorotannin as key barriers to the use of brown seaweed as a substrate for anaerobic systems, the adaptation of operating conditions to favour microbial adaptation could lead to its effective use in large-scale applications.

Major Outputs

Hierholtzer, A. and Akunna, J.C. "Modelling start-up performance of anaerobic digestion of saline-rich macro-algae". Submitted for platform presentation at: *13th World Congress on Anaerobic Digestion, Santiago de Compostela, Spain, 25th-28th June 2013*.

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List of Symbols and Nomenclature

$f_{ac,aa}$: Yield of acetate on amino acids

$f_{ac,su}$: Yield of acetate on sugars

$f_{bu,aa}$: Yield of butyrate on amino acids

$f_{bu,su}$: Yield of butyrate on sugars

$f_{ch,xc}$: Yield of carbohydrates on composites

$f_{fa,li}$: Yield of long chain fatty acids on lipids

$f_{h2,aa}$: Yield of hydrogen on amino acids

$f_{h2,su}$: Yield of hydrogen on sugars

$f_{li,xc}$: Yield of lipids on composites

$f_{pro,su}$: Yield of propionate on sugars

$f_{pr,xc}$: Yield of proteins on composites

$f_{sI,xc}$: Yield of soluble inerts on composites

$f_{va,aa}$: Yield of valerate on amino acids

$f_{xI,xc}$: Yield of particulate inerts on composites

$f_{pro,aa}$: Yield of propionate on amino acids

$I_{acetate}$: Overall inhibition function for acetate uptake

$I_{cations}$: Inhibition function for cations

$I_{dec,xac}$: Decay rate of acetate degraders

$I_{dec,xac,lam}$: Decay rate of phlorotannin tolerant acetate degraders

I_{h2}: Inhibition function for hydrogen

$I_{IN,lim}$: Inhibition function for nitrogen limitation

I_{Na^+}: Inhibition function for sodium ions

$I_{NH3,Xac}$: Inhibition function for ammonia on acetate degraders

I_{pH}: Inhibition function for pH

$K_{dec,Xaa}$: First order decay rate of amino acids degraders

$K_{dec,Xac}$: First order decay rate of acetate degraders

$K_{dec,Xac,lam}$: First order decay rate of phlorotannin tolerant acetate degraders

$K_{dec,Xc4}$: First order decay rate of butyrate and valerate degraders

$K_{dec,Xfa}$: First order decay rate of long chain fatty acids

$K_{dec,Xh2}$: First order decay rate of hydrogen degraders

$K_{dec,Xpro}$: First order decay rate of propionate degraders

$K_{dec,Xsu}$: First order decay rate of sugars degraders

K_{dis}: Disintegration rate

$K_{hyd,ch}$: Hydrolysis rate for carbohydrates

$K_{hyd,li}$: Hydrolysis rate for lipids

$K_{hyd,pr}$: Hydrolysis rate for proteins

$K_{Ih2,c4}$: Half inhibitory concentration of hydrogen for butyrate and valerate degraders

$K_{Ih2,fa}$: Half inhibitory concentration of hydrogen for long chain fatty acids degraders

$K_{Ih2,pro}$: Half inhibitory concentration of hydrogen for propionate degraders

$K_{Inh3,ac}$: Half inhibitory concentration of ammonia for acetate degraders

K_{I,Na^+}: Half inhibitory concentration of sodium ions

$K_{I,Mg^{2+}}$: Half inhibitory concentration of magnesium ions

$K_{I,Ca^{2+}}$: Half inhibitory concentration of calcium ions

K_{I,K^+}: Half inhibitory concentration of potassium ions

$K_{I,lam}$: Half inhibitory constant of phlorotannin on acetate uptake

$K_{I,lam,xac}$: Half inhibitory concentration of phlorotannin on normal acetate degraders

$K_{I,lam,xac,lam}$: Half inhibitory concentration of phlorotannin on tolerant acetate degraders

K_{La}: Gas-liquid transfer coefficient

$K_{m,aa}$: Maximum specific uptake rate for amino acids

$K_{m,ac}$: Maximum specific uptake rate for acetate

$K_{m,ac,lam}$: Maximum specific uptake rate for phlorotannin tolerant acetate degraders

$K_{m,c4}$: Maximum specific uptake rate for butyrate and valerate

$K_{m,fa}$: Maximum specific uptake rate for long chain fatty acids

$K_{m,h2}$: Maximum specific uptake rate for hydrogen

$K_{m,pro}$: Maximum specific uptake rate for propionate

$K_{m,su}$: Maximum specific uptake rate for sugars

$K_{s,aa}$: Half saturation value for amino acids uptake

$K_{s,ac}$: Half saturation value for acetate uptake

$K_{s,ac,lam}$: Half saturation value for phlorotannin tolerant acetate uptake

$K_{s,c4}$: Half saturation value for butyrate and valerate uptake

$K_{s,fa}$: Half saturation value for long chain fatty acids uptake

$K_{s,h2}$: Half saturation value for hydrogen uptake

$K_{s,pro}$: Half saturation value for propionate uptake

$K_{s,su}$: Half saturation value for sugars uptake

$K_{s,IN}$: Half saturation value for inorganic nitrogen uptake

N_{aa}: Nitrogen content of amino acids

N_{bac}: Nitrogen content of biomass

N_I: Nitrogen content of inerts

N_{Xc}: Nitrogen content of composites

S_{CAT}: Cations concentration

S_{an}: Anions concentration

S_{gas_h2}: Soluble concentration of hydrogen gas

S_{gas_ch4}: Soluble concentration of methane gas

S_{gas_co2}: Soluble concentration of carbon dioxide gas

S_{hva}: Soluble concentration of dissociated valerate

S_{hbu}: Soluble concentration of dissociated butyrate

S_{hpro}: Soluble concentration of dissociated propionate

S_{hac}: Soluble concentration of dissociated acetate

S_{hco3}: Soluble concentration of bicarbonate

S_{nh3}: Soluble concentration of ammonia

S_{Na}^{+}: Soluble concentration of sodium ions

S_{Mg}^{2+}: Soluble concentration of magnesium ions

S_{Ca}^{2+}: Soluble concentration of calcium ions

S_{K}^{+}: Soluble concentration of potassium ions

S_{su}: Soluble concentration of sugars

S_{aa}: Soluble concentration of amino acids

S_{fa}: Soluble concentration of long chain fatty acids

S_{va}: Soluble concentration of valerate

S_{bu}: Soluble concentration of butyrate

S_{pro}: Soluble concentration of propionate

S_{ac}: Soluble concentration of acetate

S_{h2}: Soluble concentration of hydrogen

S_{ch4}: Soluble concentration of methane

S_{IC}: Soluble concentration of inorganic carbon

S_{IN}: Soluble concentration of inorganic nitrogen

S_I: Soluble concentration of inerts

S_{lam}: Soluble concentration of phlorotannin

X_{xc}: Particulate concentration of composites

X_{ch}: Particulate concentration of carbohydrates

X_{pr}: Particulate concentration of proteins

X_{li}: Particulate concentration of lipids

X_{su}: Particulate concentration of sugars

X_{aa}: Particulate concentration of amino acids

X_{fa}: Particulate concentration of long chain fatty acids

X_{c4}: Particulate concentration of butyrate and valerate

X_{pro}: Particulate concentration of propionate

X_{ac}: Particulate concentration of acetate degraders

$X_{ac,lam}$: Particulate concentration of phlorotannin tolerant acetate degraders

X_{h2}: Particulate concentration of hydrogen

X_I: Particulate concentration of inerts

Y_{aa}: Yield of amino acids uptake

Y_{ac}: Yield of acetate uptake

$Y_{ac,lam}$: Yield of acetate uptake for phlorotannin tolerant degraders

Y_{c4}: Yield of butyrate and valerate uptake

Y_{fa}: Yield of long chain fatty acids uptake

Y_{h2}: Yield of hydrogen uptake

Y_{pro}: Yield of propionate uptake

Y_{su}: Yield of sugars uptake

Chapter 1
Introduction and problem statement

This chapter presents the general perspective of this work and states the research problematic that is developed and investigated in the study. It also introduces aims and objectives and outlines the methodology adopted in addition to the structure of the thesis.

1.1. General perspective and problem statement

Anaerobic digestion is commonly recognised as a sustainable method for converting organic matter to energy (Mata-Alvarez 2003). The underlying principles of anaerobic digestion are well established and advances in process control have put the method at the forefront of renewable energy solutions (Clarke and Alibardi 2010). The interest in anaerobic digestion has been further strengthened by regulatory incentives and the forecasted energy crisis with ramifications beyond natural resources exhaustion, fossil fuels shortages and geopolitical trends. However, the technology is still associated with high initial costs and a long-term return on investment. A systematic solution to mitigate the latter is to increase the net energy production and thus, favour the economic appraisal of anaerobic digesters. Consequently, innovative technological solutions associated with specific operational and feedstock preparation strategies have been successfully developed for the enhancement of biogas yields. Other

solutions have seen the combination of successive aerobic and anaerobic treatments involving the conversion of the resulting liquid fraction of anaerobic digestion to fertiliser. With the development of 'high-rate' systems and the generalisation of the technology, some challenges common to other conversion processes have emerged. The scarcity of appropriate sources of organic matter has been found to be critical because of the competition existing with alternative treatment solutions, inadequate waste segregation practices and fiscal inducements. To this extent, the use of purposely grown energy crops has been generally accepted as an effective solution to securing sustainable and sufficient sources of organic matter for the generation of biofuels (Directive 2009/28/EC). However, the overwhelming use of energy crops to the detriment of food feedstocks has been found to often contravene the environmental benefits originally planned and has resulted in the increase of global food prices. The European Commission has thus recently proposed to put a cap of 5% on the food-based biofuel allowed in the renewable energy used in transport, with the aim of encouraging energy production from waste, algae and alternative feedstocks rather than from food crops (European Commission 2012).

Whilst the need for substituting feedstock sources to promote anaerobic digestion is acknowledged, little has been done for the improvement of existing anaerobic systems relying on a single source of material often varying in both quality and availability. Numerous studies (Cecchi *et al.* 1996; Edelmann *et al.* 2000; Sosnowski *et al.* 2003; Astals *et al.* 2010; Chen *et al.* 2010; Neira and Jeison 2010; Fang *et al.* 2011; Costa *et al.* 2012) have focussed on the simultaneous digestion of two or more substrates, yet none of these studies have considered the addition of a co-substrate as a means of sustaining optimum conversion rates in conditions of seasonal feedstock variability. A typical example is the anaerobic digestion of vegetable and

fruit residues, the availability of which is dependent upon both climatic conditions and the season of the year.

Marine biomass represents a unique and diverse reserve of organic matter. Brown seaweed is of particular interest because of its abundance on the sublittoral zone of the British coastline and appealing conversion rates in anaerobic systems (Chynoweth *et al.* 1987; Hanssen *et al.* 1987; Adams *et al.* 2011b). Another advantage of brown seaweed is that immersion in seawater results in high biomass productivity with a corresponding improvement of the feedstock sustainability in comparison with purposely grown energy crops requiring a combination of water, fertiliser and extensive acreage. Major challenges associated with the anaerobic digestion of brown seaweed are the effect of inherently high content of salts and a unique class of phenolic compounds on the microbial consortium found in anaerobic systems. Therefore, the establishment and study of the limitations relating to the use of brown seaweed as a complementary source of organic matter in anaerobic digesters forms the core of this research project. Light metal salts, particularly sodium, are well-known inhibitors of anaerobic systems and excessive concentrations have been reported to cause serious microbial inhibition (Rinzema *et al.* 1987; Liu and Boone 1991; Feijoo *et al.* 1995; Lefebvre and Moletta 2006). However, bacteria are versatile organisms and have been found capable of adapting to severe environmental changes such as those resulting from sodium ion accumulation (Oren 2002b). Yet, very little has been reported on microbial adaptation and the effect of fluctuating levels of salts, a likely occurrence in anaerobic co-digestion systems. Polyphenolic compounds are another category of potential inhibitors and they typically affect anaerobic digestion through interactions with cell membranes and interference with microbial metabolism (Scalbert 1991; McDonnell and Russell 1999). However, very little is known about

the impact and mode of action of the peculiar type of phenols found in brown seaweed on anaerobes. There is also need to understand the relationship between the degree of polymerisation of the compound and the associated inhibitory effect as the toxicity of various types of phenols has been observed to be a function of their molecular weight (Gilbert *et al.* 1990; Nagayama *et al.* 2002).

One of the barriers to the uptake of anaerobic digestion is the lack of appropriate and reliable tools to predict system behaviour and possible outcomes from changes in environmental conditions or operational parameters. Anaerobic systems are highly complex and dynamic, owing to the fact that the degradation of organic matter and its subsequent transformation to biogas occurs through simultaneous biochemical and physico-chemical processes involving heterogeneous guilds of microorganisms. The modelling of anaerobic digestion started with restricted models focusing on the numerical representation of specific processes. Such mathematical models, though significant for research purposes, could only find limited practical use. The advent of more complex models, representing the multiple stages involved in anaerobic digestion, resulted in their potential application in process engineering including the design, control, operation and optimisation of anaerobic systems. Complex models require the calibration of numerous variables and coefficients, which render their use tedious. In an effort to both synthesise the latest progress made in the modelling of anaerobic digestion and to create a generic modelling platform, the International Wastewater Association Task Group for Mathematical Modelling of Anaerobic Digestion Processes developed the Anaerobic Digestion Model No.1 (Batstone *et al.* 2002). The model rapidly became a reference point and has witnessed a myriad of applications ranging from the anaerobic digestion of organic waste (Derbal *et al.* 2009), domestic wastewater (Feng *et al.* 2006) and grass silage (Koch *et*

al. 2010) to serving as a basis for the representation of carbohydrate degradation by human colonic microbiota (Muñoz-Tamayo *et al.* 2010). However, the mechanisms of inhibition adopted in the original model structure do not appropriately address the potentially detrimental effects of inhibitors commonly found in brown seaweed such as sodium ions or phenolic compounds. Moreover, the model does not currently take into consideration the different disintegration and hydrolysis rates occurring during the simultaneous digestion of two or more substrates and hence cannot be used to model co-digestion at varying substrate ratios. The implementation of additional mathematical functions representing specific inhibitors and the prognostic use of the model during the anaerobic co-digestion of brown seaweed is hence proposed in this work. There is also a need to assess the importance of the model parameters on the target outputs, and hence rationalise the assumptions made on their values in order to promote the use of dynamic models for the control and optimisation of anaerobic systems.

1.2. Research aim and objectives

The overall aim of this work is to investigate the potential of brown seaweed as both an additional and alternative source of organic matter for anaerobic digesters using a combination of experimental and modelling approaches.

The specific objectives of the study are as follows:

- To critically analyse the Anaerobic Digestion Model No.1 (ADM1) and establish the relative importance of the model variables using parametric sensitivity analysis methods.

- To modify and calibrate the ADM1 for the prediction of the effects of sodium ions on the anaerobic digestion process.

- To evaluate and model the impact and mode of action of phenolic compounds contained in brown seaweed on anaerobic digestion processes and microorganisms.

- To establish operational guidelines for the co-digestion of seaweed with non-saline feedstock.

1.3. Dissertation outline

The thesis is articulated around 13 Chapters including this introductory section. Chapters 7 to 12 present experimental results, and each Chapter contains an introduction followed by a brief explanation of the methodology used, results and discussion, and ends with a conclusion. **Chapter 2** reviews the general concepts of anaerobic digestion and introduces important factors affecting the process. It includes information on the most popular types of anaerobic systems and their performances. **Chapter 3** introduces the characteristics of brown seaweed and the factors likely to affect its potential use as a feedstock for anaerobic digestion. **Chapter 4** presents an overview of the mathematical modelling of anaerobic digestion and includes detailed information on the structure of the Anaerobic Digestion Model No.1, which is used throughout this work. **Chapter 5** summarises the themes reviewed through Chapters 2 to 4 and supports the experimental and modelling approaches used in the subsequent sections of the thesis. **Chapter 6** presents the material and methods used in experimental Chapters along with information on the accuracy, repeatability and precision of all analytical methods. **Chapter 7** compares the results of two sensitivity

analysis techniques for the determination of the most influencing model parameters on the performance of anaerobic digesters during start-up. **Chapter 8** discusses the effect of increasing sodium ion concentrations on anaerobic systems and suggests modification to the Anaerobic Digestion Model No.1 to consider the inhibitory effect of sodium ions on acetoclastic methanogens. **Chapter 9** develops the results obtained in the previous section by investigating and modelling the factors affecting the adaptation of anaerobic systems to increasing levels of salinity. **Chapter 10** illustrates the impact and mode of action of phenolic compounds extracted from brown seaweed on mixed microbial cultures found in anaerobic systems. **Chapter 11** extends the structure of the Anaerobic Digestion Model No.1 to include a function that considers the effect of phenolic compounds on acetoclastic methanogens, as observed experimentally in a laboratory-scale anaerobic digester. **Chapter 12** validates operational strategies for the optimisation of the anaerobic co-digestion of brown seaweed whilst **Chapter 13** provides a summary of results found in experimental Chapters, a general reflexion on this work, conclusions and future perspectives.

Chapter 2
Anaerobic digestion

This chapter provides a general overview of the steps involved in the anaerobic digestion of organic matter and introduces important factors affecting the process. It also includes information on the most common types of anaerobic systems and the impact of their design on process performances.

2.1. Anaerobic digestion fundamentals

The anaerobic digestion of organic matter is a natural process that has been witnessed and reported since time immemorial. It is believed that, in ancient times, the phenomenon was mainly observed through the ignition of methane released by the anaerobic degradation of organic matter present in wet soil (Abbasi *et al.* 2012). The formation of methane by strictly anaerobic microorganisms was later observed by Volta in 1776 and confirmed by many researchers (Hamer 2010). For the past decades, anthropogenic anaerobic digestion has seen significant developments, often driven by successive energy crises and society's needs, but the discipline is nowadays mature enough to be considered as a reliable method for the generation of renewable energy through biogas production (De Baere 2000; Mata-Alvarez 2003). Anaerobic digestion is a multidisciplinary area because of the diverse processes involved and therefore, relies on the advances made in microbiology, biochemistry, chemical and environmental engineering (Clarke and Alibardi 2010). A vast majority of mechanisms associated with the anaerobic degradation of organic matter are

biochemical, as they occur at cellular level and involve varied microbial populations. Mechanisms that are non-biologically mediated, such as acid-base reactions, are considered as physico-chemical processes and are of prime importance in the performance of anaerobic systems. These are discussed in the following sections.

2.1.1. Biochemical processes

The main biochemical steps during anaerobic digestion are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Pohland 1992) as shown in Figure 2.1.

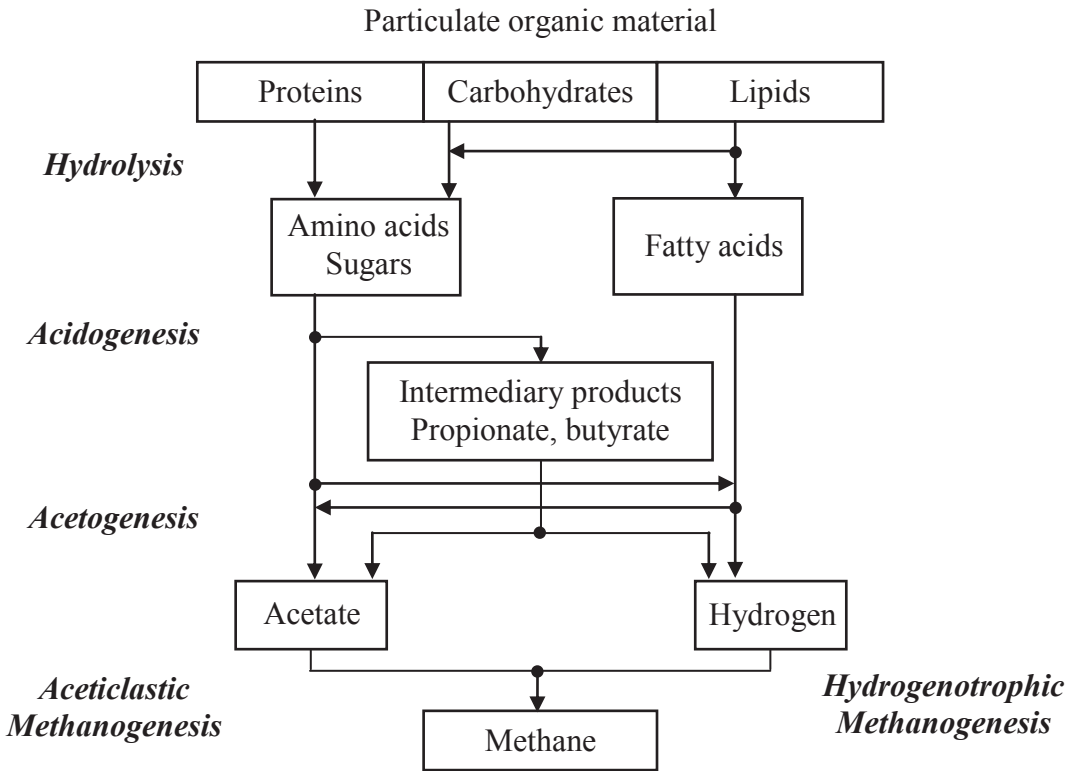

Figure 2.1. Scheme of the anaerobic biodegradation steps of complex organic matter (adapted from Gujer and Zehnder 1983).

These consecutive steps are accomplished by representatives of four main metabolic microbial groups respectively: hydrolytic-fermentative bacteria, proton-reducing

acetogenic bacteria, hydrogenotrophic methanogens and aceticlastic methanogens (Zinder *et al.* 1984). Hydrolysis is the first step of the anaerobic biodegradation of complex organic material and results in the breakdown of proteins, carbohydrates and lipids into monomeric compounds such as amino acids, sugars and fatty acids. During acidogenesis, these compounds are further transformed into hydrogen, formate, volatile fatty acids, ethanol, ketones or lactic acid and other organic products usually named intermediary compounds, but can also be transformed directly to acetate or hydrogen by specific microorganisms. These intermediary compounds are then converted to acetate and hydrogen during acetogenesis. The last step involves the conversion of acetate and hydrogen to methane through aceticlastic methanogenesis and hydrogenotrophic methanogenesis respectively. Other processes involve acetogenic respiration of bicarbonates by homoacetogenic bacteria and oxidation of acetate and hydrogen by sulphate and nitrate reducing bacteria (Mata-Alvarez 2003). A non-exhaustive list of microorganisms involved in the different degradation steps can be seen in Table 2.1.

Hydrolysis, as the first process occurring during anaerobic digestion, is also considered to be the rate-limiting step during the degradation of complex organic matter. The slow rate of hydrolysis is a consequence of both the difficulties associated with the chemical decomposition of complex polymeric substances, and the limited accessibility of hydrolytic microorganisms to the solid matter (Eastman and Ferguson 1981). The process can be promoted through an increase of the available specific surface to the medium by mechanical, biological or chemical pretreatments aimed at breaking the biopolymeric chains into soluble monomeric or oligomeric components (Delgenès *et al.* 2003).

Table 2.1. Microorganisms involved in the different steps of anaerobic digestion (adapted from Stronach *et al.* 1986)

Degradation process	Bacterial group	Type of conversion and associated bacteria
Hydrolysis	Hydrolytic bacteria	*Clostridium, Proteus vulgaris, Peptococcus, Bacteriodes, Bacillus* convert proteins to peptides and amino acids *Clostridium, Acetovibrio celluliticus, Staphylococcus, Bacteriodes* transform carbohydrates to soluble sugars *Clostridium, Micrococcus, Staphylococcus* convert lipids to higher fatty acids or alcohol and glycerol
Acidogenesis	Fermentative bacteria	*Lactobacillus, Escherichia, Staphylococcus, Bacillus, Pseudomonas, Desulfovibrio, Selenomonas, Sarcina, Veillonella, Streptococcus, Desulfobacter, Desulforomonas* transform amino acid to fatty acids, acetate and ammonia *Clostridium, Eubacterium limosum, Streptococcus* convert sugars to intermediary fermentation products
Acetogenesis	Acetogenic bacteria	*Clostridium, Syntrophomonas wolfei* transform higher fatty acids or alcohols to hydrogen and acetate *Syntrophomonas wolfei, Syntrophomonas wolinii* convert volatile fatty acids and alcohols to acetate and hydrogen
Methanogenesis	Acetoclastic and hydrogenophilic methanogenic bacteria	*Methanobacterium, Methanobreviacterium, Methanoplanus, Methanospirillum* transform hydrogen and carbon dioxide to methane *Methanosaeta, Methanosarcina* convert acetate to methane and carbon dioxide

It has also been found that the hydrolysis rate depends strongly on the origin and the potential adaptation of the anaerobic microorganisms to a specific substrate (Gavala *et al.* 1999). This is supported by the conceptual model that hydrolytic microorganisms attach to a particle, produce enzymes in the surrounding area and benefit from the soluble products released by the enzymatic reaction (Vavilin *et al.* 1996). The efficiency of the later mechanism is likely to be dependent on the predominant type of bacteria found in the system, which is strongly influenced by the composition of the substrate being digested. Hydrolysis can be inhibited by high levels of amino acids and sugars (Sanders *et al.* 1999; Kadam *et al.* 2004) due to the obstruction of enzymatic production and activity (Garcia-Heras 2003). Hydrolysis can also be inhibited by high concentrations of long-chain and volatile fatty acids, hydrogen, ammonia and too acidic or alkaline pH values (Batstone *et al.* 2002).

Immediately following hydrolysis, acidogenesis is usually the quickest step during the anaerobic digestion of complex organic material (Vavilin *et al.* 2008). The process, sometimes referred to as a fermentation step, is defined as an anaerobic acid-producing microbial process without additional electron acceptor or donor which relies on the action of both obligate and facultative anaerobic bacteria (Gujer and Zehnder 1983). Products from hydrolysis are fermented to form short-chain (C1-C5) volatile fatty acids, specifically lactic, propionic, butyric and valeric acids (Abbasi *et al.* 2012). The reaction can occur at high hydrogen concentrations and at high biomass yields because it does not require an additional electron acceptor and free energy yields are usually high (Batstone *et al.* 2002). The fermentation of glucose can also yield ethanol whilst the degradation of amino acids results in the production of ammonia (Garcia-Heras 2003). Because acidogenesis is a rapid process and anaerobic digestion is the result of a sequence of reactions, it is important to ensure a balance

between the rates of the different steps involved in the overall anaerobic biodegradation (Vavilin *et al.* 2008). The importance of a well-balanced system is often illustrated through the accumulation of short-chain volatile fatty acids that may decrease the pH to values where methane-forming bacteria are negatively impacted.

Acetate, being partly produced during fermentation, can also result from acetogenesis. The latter is often considered simultaneously with hydrogenotrophic methanogenesis, since microorganisms involved in hydrogen-producing acetogenesis and hydrogen-utilising methanogenesis grow in a syntrophic co-culture (Garcia-Heras *et al.* 2003). Low molecular weight volatile fatty acids are converted into acetate, hydrogen and carbon dioxide gases, but the process only occurs in a narrow range of hydrogen concentrations (Batstone *et al.* 2002), thus explaining why hydrogenotrophic methanogenesis is essential to maintain hydrogen at suitable levels for the conversion of volatile fatty acids.

The final step in anaerobic digestion is the conversion of intermediary compounds to methane. The typical methanogenic reactions arising during the conversion process are shown in Table 2.2. Methanogenesis takes place through two processes. As mentioned above, hydrogenotrophic methanogens produce methane from the use of hydrogen and carbon dioxide and are cross-feeding with hydrogen-producing acetogens. The other pathway for methane production is the conversion of acetate by acetoclastic methanogens that also produce carbon dioxide as a by-product. The later mechanism accounts for most of the methane being produced during the anaerobic biodegradation process (Pohland 1992). Both hydrogenotrophic and acetoclastic methanogens belong to the domain archaea (Demirel and Scherer 2008), that used to be considered as a form of bacteria, but are now recognised as a distinctive group of microorganisms (Wharton 2002). Methanogens are characterised

by long generation times, i.e. the period of time required to double their number, and with certain types of substrates, methanogenesis might become the rate-limiting step instead of hydrolysis (Bouallagui *et al.* 2005).

Table 2.2. Methanogenic reactions during anaerobic digestion (adapted from Chynoweth 1996).

Hydrogen	$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$
Acetate	$CH_3COOH \rightarrow CH_4 + CO_2$
Formate	$4\ HCOOH \rightarrow CH_4 + 3\ CO_2 + 2\ H_2O$
Methanol	$4\ CH_3OH \rightarrow 3\ CH_4 + CO_2 + 2\ H_2O$
Carbon monoxide	$4\ CO + 5\ H_2O \rightarrow CH_4 + 3\ H_2CO_3$
Trimethylamine	$4(CH_3)_3N + 6\ H_2O \rightarrow 9\ CH_4 + 3\ CO_2 + 4\ NH_3$
Dimethylamine	$2(CH_3)_2NH + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 2\ NH_3$
Monomethylamine	$4(CH_3)NH_2 + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 4\ NH_3$
Methyl mercaptans	$2(CH_3)_2S + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 2\ H_2S$
Metals	$4\ Me^{+} + 8\ H^{+} + CO_2 \rightarrow 4\ Me^{++} + CH_4 + 2\ H_2O$

Methanogens are sensitive to alkaline or acid pH and obligate archaea anaerobes are strongly inhibited below a pH of 6.2 (Demirel and Scherer 2008). A shift of pH towards acidic values triggered by volatile fatty acids accumulation may be both a precursor and a consequence of methanogenesis inhibition. The most common inhibitors of methanogenesis in anaerobic systems are free ammonia and hydrogen (Batstone *et al.* 2002). However, the fate of acetotrophic methanogens in anaerobic digesters seem to be significantly impacted by the source and type of inoculum, the success of the start-up phase and operational parameters, such as the hydraulic

retention time, temperature or volatile fatty acids fluctuations (Demirel and Scherer 2008).

2.1.2. Physico-chemical processes

The physico-chemical processes occurring in anaerobic systems are mainly ion association/dissociation, liquid-gas transfer and solids precipitation/solubilisation (Batstone *et al.* 2002). Acid-base reactions are of great importance during anaerobic biodegradation, since the toxicity of some compounds towards anaerobic microorganisms is dictated by their ion association/dissociation reactions with hydrogen and hydroxide ions (Chen *et al.* 2008). This is the case with sulphide that can be found in the unionised form of hydrogen sulphide (H_2S) and as hydrogen sulphide ion (HS^-). Another example is inorganic nitrogen that can be found as free ammonia (NH_3) and ammonium ions (NH_4^+). Similarly, liquid-gas transfer is an important phenomenon since it impacts upon biological processes through hydrogen concentration. The amount of soluble carbon dioxide can also influence pH by the formation of carbonic acid in water. Liquid-gas transfer occurs when a condensed liquid phase equilibrates with a gas phase resulting in some concentration of the gaseous compound being dispersed in the liquid phase (Ozima and Podosek 2002). The solubility of gases depends mainly on environmental factors, such as temperature and pressure (Battino and Clever 1966). In anaerobic systems, hydrogen and methane are considered as having low solubility, whereas ammonia and carbon dioxide are considered highly soluble.

Solids precipitation in anaerobic digesters mainly concerns systems with high inputs of struvite or calcium, such as that found in paper industries (Huster *et al.* 1991) or when lime is used as a neutralising agent. Excessive concentrations of

calcium can lead to the precipitation of carbonate and phosphate, which may result in the scaling of biomass and a reduced methanogenic activity (Keenan *et al.* 1993).

2.2. Operational parameters and design criteria for anaerobic digestion

2.2.1. Inoculum characteristics

Anaerobic digestion requires the presence of several types of microorganisms. The use of an inoculum containing the required microbial populations is thus of great importance for the anaerobic degradation to proceed (Angelidaki and Sanders 2004). Active anaerobic microorganisms are often inoculated into anaerobic systems during the start-up period in order to reduce the lag-phase associated with the development of an appropriate microbial consortium. The type of microorganisms found in the inoculum will depend on the operational conditions and type of substrate used in the system they originate from, and the environment they will be later subjected to will determine the fate of the different microbial groups. The selection of an appropriate source of inoculum is essential, as the success of the start-up phase is crucial to reach stable operation (Fernández *et al.* 2001). It is particularly important with the treatment of substrates characterised by significant levels of inhibitory compounds. Aspé *et al.* (1997) compared the performances of two different inocula during the anaerobic treatment of fishery wastewater and found that the inoculum originating from marine sediments adapted better and faster to the specific inhibitors associated with fishery wastewater when compared to microorganisms obtained from pig manure. The use of an adapted source of inoculum also positively impacted the methane yields measured during the anaerobic digestion of macroalgae (Schamm and Lehnberg 1984), but was not found beneficial in a system in which hydrolysis was the rate-limiting step (Costa *et al.* 2012). The latter observation is supported by the fact that methanogens are

usually the most sensitive microorganisms to environmental changes and the selection of an adapted source of inoculum is expected to mainly affect methanogenesis by providing the adequate archaea in sufficient numbers for successful conversion. The concentration of inoculum added in an anaerobic batch system is usually dependent on the substrate concentration but should be sufficient to avoid acidification and aid process stability (Hansen *et al.* 2004).

2.2.2. Substrate characteristics

The key factors influencing both the rates and yields of the successive anaerobic digestion processes rely on the characteristics of the substrate. The composition and potential inhibitory substances associated with a specific substrate will determine its successful anaerobic biodegradation. Moreover, biochemical composition and concentrations of inhibitory substances may be dependent on season, origin and species for organic residues. The specific methane potential is also dependent of the amount of carbohydrates, proteins and lipids defining the substrate. Substrates rich in carbohydrates tend to be rapidly converted to volatile fatty acids, which can lead to acidification and process inhibition (Jiang *et al.* 2012). Lipid-rich substrates are considered as easily biodegradable, but might induce inhibition because of the accumulation of long chain fatty acids during the hydrolysis of neutral lipids (Labatut *et al.* 2011). Whilst proteinaceous substrates might result in high ammonia levels being produced by the anaerobic degradation of the nitrogenous matter (Chen *et al.* 2008) and high lignin content is likely to reduce the hydrolysis rate and consequently the extent of degradation (Sanders *et al.* 2003). The proportions of carbon and nitrogen found in a substrate are also decisive with regards to its successful biodegradation. Substrates containing high amounts of carbon when compared to

nitrogen might be undesirable because the low levels of nitrogen are consumed rapidly by the methanogens and subsequent nitrogen-limitation is likely to impair bacterial growth. Whereas, inhibition by ammonia might be a consequence of high nitrogen levels (Abbasi *et al.* 2012). The elemental composition of a substrate is a key factor, since it can also be used as an indicator of potential sulphide inhibition by measuring the levels of sulphur in the organic matter. Finally, some substrates are characterised by inherently high levels of inhibitors, such as benzenes, phenols, aromatic compounds and light or heavy metals that may impair their anaerobic degradation (section 2.3.2).

2.2.3. Volatile fatty acids, alkalinity and pH

The concentration of volatile fatty acids, alkalinity and ammonia are dependent variables as they define the buffering capacity and hence, pH values of an anaerobic system. pH is commonly considered as the most critical parameter to be controlled in anaerobic digesters and is often used as an indicator of system stability (Killilea *et al.* 2000; Bouallagui *et al.* 2003). The optimum pH for anaerobic digestion is reported to be around neutral, at which the yield of most biochemical processes is favoured (Verrier *et al.* 1987; Liu *et al.* 2008). If pH is an important parameter during anaerobic digestion, its value is mainly dependent on the buffering capacity of the system where several other reactions are involved and therefore, cannot be considered as a sole indicator of process stability. Cecchi *et al.* (2003) suggest that when pH variations are observed, the reaction medium has already lost its stability and important changes in pH should therefore be considered as an indicator of process failure rather than an indicator of process instability. One important parameter affecting pH is the acid-neutralising capacity of the system or alkalinity. The alkalinity of the medium,

defining its ability to resist pH changes, is a result of the presence of hydroxides, carbonates and bicarbonates (Cecchi *et al.* 2003). In a well-operated anaerobic system, reaction products such as volatile fatty acids are consecutively transformed until methanogenesis without accumulation and thus, results in stable pH values (Figure 2.1). In an unbalanced system, with an accumulation of volatile fatty acids resulting from the slow growing capacity of methanogens, the alkalinity of the medium will become particularly important in attempts to maintain pH values near to neutral and to prevent a possible system failure. The concentration of volatile fatty acids is generally suggested as a process indicator, since it is the main pre-methanogenic intermediate (Molina *et al.* 2009; Boe *et al.* 2010). Volatile fatty acids, as the main intermediary compounds before methanogenesis, will tend to accumulate during an unbalanced development of the trophic chain. The accumulation of these acids will eventually lead to the decrease of pH at a rate which depends on both their concentrations and the alkalinity within the system. Volatile fatty acids can also have a toxic effect on microorganisms. Particularly, undissociated species are reported as more toxic because of their ability to diffuse to the inner parts of the cell with propionic and butyric acid being most inhibitory (Mata-Alvarez 2003). Because of the several simultaneous processes involved during anaerobic digestion, a combination of different parameters might be required to assess the performances of an anaerobic digester (Boe *et al.* 2010). In this view, the ratio between volatile fatty acids and alkalinity is often considered as a suitable measure of process stability with values between 0.4 and 0.8 being favourable (Rao and Singh 2004; Bouallagui *et al.* 2009).

2.2.4. Organic loading rate and hydraulic retention time

The quantity of substrate being introduced in an anaerobic reactor during a given period of time is defined as the organic loading rate of the system. It is mainly dependent of the type of substrate, but also on the reactor size and hydraulic retention time or temperature (Garcia-Heras 2003). The amount of organic matter loaded into the system must be carefully chosen to maintain its stability. The rapid increase of the loading rate is likely to result in the build-up of volatile fatty acids, which in turn might lead to process failure. In practice, the organic loading rate is defined prior to the design of an anaerobic system together with the hydraulic retention time, which are the most commonly used parameters to determine the volume of a digester (Linke 2006). The hydraulic retention time is a measure of the period that a fluid element spends in an anaerobic digester. Its value should be a compromise between the time necessary for the microbial consortium to degrade most of the organic matter and a period of time that should be short enough to guarantee a limited reactor volume and hence, reduce construction and maintenance costs. The hydraulic retention time should also be high enough to allow the active microbial populations to remain in the reactor, especially the slow growing methanogens (Sialve *et al.* 2009). Therefore, the retention time is sometimes considered to be an indication of reactor efficiency (Abbasi *et al.* 2012). Because the hydraulic retention time is a function of the substrate flow rate in a reactor with a given volume, it is often representative of the loading rate applied. The impact of a given inhibitory compound concentration applied at a low hydraulic retention time would hence, be greater when compared to the same concentration applied at high retention times due to the detrimental effect of most inhibitors on the growth rate of microorganisms (Ergüder *et al.* 2003).

2.2.5. General aspects of co-digestion

The anaerobic co-digestion concept involves the treatment of two or more substrates in a single system (Hartmann *et al.* 2003). The simultaneous anaerobic degradation of different substrates is not a new concept, with early references appearing in the late seventies, but the approach has seen a tremendous increase in its application during the past few years (Mata-Alvarez *et al.* 2009). As its main benefits, co-digestion has been reported to enhance the overall process stability through the improvement of the system buffering capacity, synergistic effect of microorganisms or the addition of any amendment which a substrate by itself may lack (Nayono *et al.* 2010; Khalid *et al.* 2011; Labatut *et al.* 2011). The success of co-digestion usually lies in the harmonisation of parameters between the sources of substrates as shown in Figure 2.2. Of particular interest is the dilution of potential toxic compounds found in one substrate through the addition of another source of organic matter. This additional substrate can guarantee an optimum loading rate and prevent the accumulation of inhibitors such as sodium or ammonia at toxic levels (Sialve *et al.* 2009; Fang *et al.* 2011). It can also be a solution for the anaerobic treatment of nutrient-deficient substrates (Neira and Jeison 2010; Gurung *et al.* 2012). The co-digestion of organic matter can also be of use in the improvement of the loading rate of a system (and hence biogas yields) when the main source of feedstock is in limited availability (Chen *et al.* 2010). This is particularly relevant for large-scale digestion plants located in geographically remote areas in which costs associated with the transportation of feedstock could be reduced and single feedstock digestion is considered unsustainable (Edelmann *et al.* 2000; Pöschl *et al.* 2010). Co-digestion can also secure the stable year-round operation of anaerobic digesters treating substrates that are seasonal by nature or during crop rotation (Gavala *et al.* 1996; Lansing *et al.* 2010).

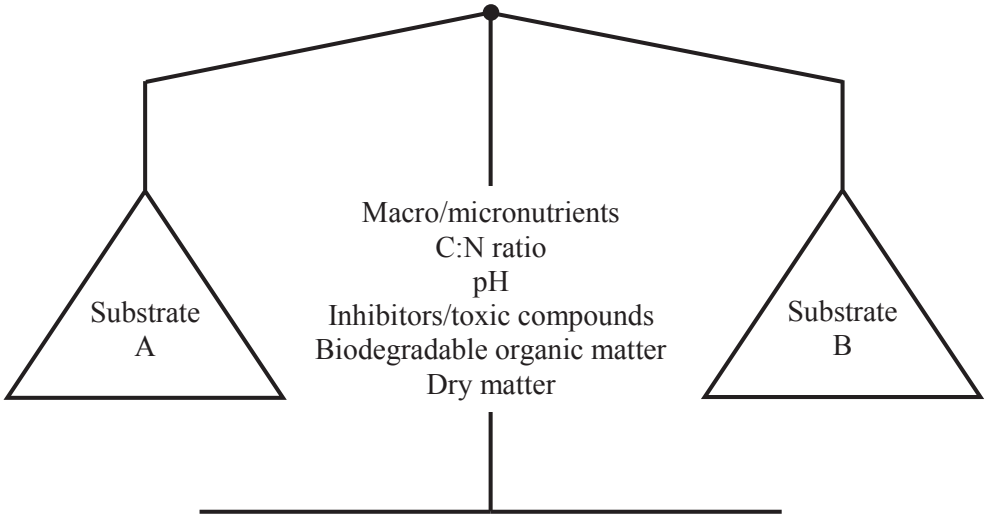

Figure 2.2. The balance of co-digestion (adapted from Hartmann *et al.* 2003).

However, some drawbacks can be associated with the addition of a new source of organic matter. The high cost of co-substrate transfer from the generation point to the digestion plant, the risk of spreading poisonous substances not originally found in the system or the impact on process yields and kinetics that could be triggered by a new substrate should all be carefully considered (Astals *et al.* 2010).

2.3. Environmental factors affecting the anaerobic digestion process

2.3.1. Nutrients

Vitamins, macro- and micro-nutrients are necessary to obtain the optimal function of anaerobic microorganisms in a degradative process. The necessary nutrients are normally found in sufficient quantities in most of the substrates, but some essential micronutrients might be lacking in anaerobic digesters operating over a long period with the same feedstock, especially energy crops or vegetable residues (Lindorfer *et al.* 2011; Jiang *et al.* 2012). Basic nutrient requirements are provided through carbon,

phosphorous and nitrogen with the latter element being the most essential and, in all likelihood the most limiting for bacterial growth and reproduction. Generically, nutrients include trace quantities of light metals such as potassium, calcium or magnesium, which are used by most of the microorganisms to maintain cell membrane integrity and regulate osmotic pressure (Angelidaki and Sanders 2004). Heavy metals are also required in order to sustain microbial metabolism and traces of heavy metals in the form of iron, zinc, cobalt, nickel or tungsten are usually found in healthy digesters (Angelidaki *et al.* 2009; Demirel and Scherer 2011). Different types of vitamins, amino-acids, purines and pyrimidines might also be a requisite to enhance microbial activity (Angelidaki and Sanders 2004). Organic carbon and nitrogen usually form the basis of biodegradable substrates. However, some required microelements are not found in sufficient quantities in digesters depending on a sole source of feedstock and are therefore added as process additives. The typical additives added in anaerobic digesters can be seen in Table 2.3. The addition of nutrients in anaerobic systems should however be carefully planned since besides the costs involved in the use of chemicals in large quantities, most of these additives become toxic after a certain threshold level and their addition does not necessarily improve system efficiency (Mata-Alvarez 2003; Wan *et al.* 2011).

Table 2.3. Common additives added in anaerobic digesters (adapted from Koch *et al.* 2011).

Additive	Example	Description
Microorganisms	Hydrolytic strains	Supplementation for the existing biological consortium. Aid the adaptation of microorganisms to new substrates or environmental conditions
Trace elements	Iron, cobalt, nickel, zinc, tungsten	Essential for the growth of microorganisms
Exchangers	Zeolites, minerals	Reduce the concentration of inhibitory or toxic substances and may supply necessary light metals
Enzymes	Cellulase, amylase, protease, xylanase	Enhance the degradation of polymers and can decrease the viscosity of the suspension

2.3.2. Toxic compounds

Inhibition of anaerobic digestion by toxic compounds can be triggered by a vast range of substances, which above a certain concentration, impact negatively the microbial consortium. The most common inhibitors reported in literature include ammonia, sulphide, long chain fatty acids, salts, heavy metals, phenolic compounds, and xenobiotics (Mata-Alvarez 2003; Chen *et al.* 2008). These compounds can be classified into two groups with toxic substances (toxicants) resulting in an adverse, but not necessary lethal effect on microorganisms and inhibitors impairing bacterial function by affecting specific targets or the overall cell kinetics and functions (Speece 1996). Examples of toxicants include long chain fatty acids, nitro-compounds and antibiotics, whereas hydrogen sulphide, ammonia or volatile fatty acids are inhibitors (Batstone *et al.* 2002). At low concentration, most inhibitors have a stimulatory effect on the overall biodegradation process. With an increase of their concentration, these substances become toxic at a level that will be dependent on environmental factors, operational parameters or adaptation of the biomass. Figure 2.3 illustrates the

different phases of the inhibition phenomenon. However, some compounds might have an immediate and irreversible lethal effect on most of the microorganisms and are categorised as biocides. Xenobiotics and some phenolic compounds used for their antiseptic and disinfectant properties can be classified in the latter category (McDonnell and Russell 1999). Strategies to reduce the effect of inhibitors include adaptation of the microbial consortium or co-digestion in means of substrate dilution resulting in decreased concentrations of inhibitors being introduced in the system (Fujishima *et al.* 2000).

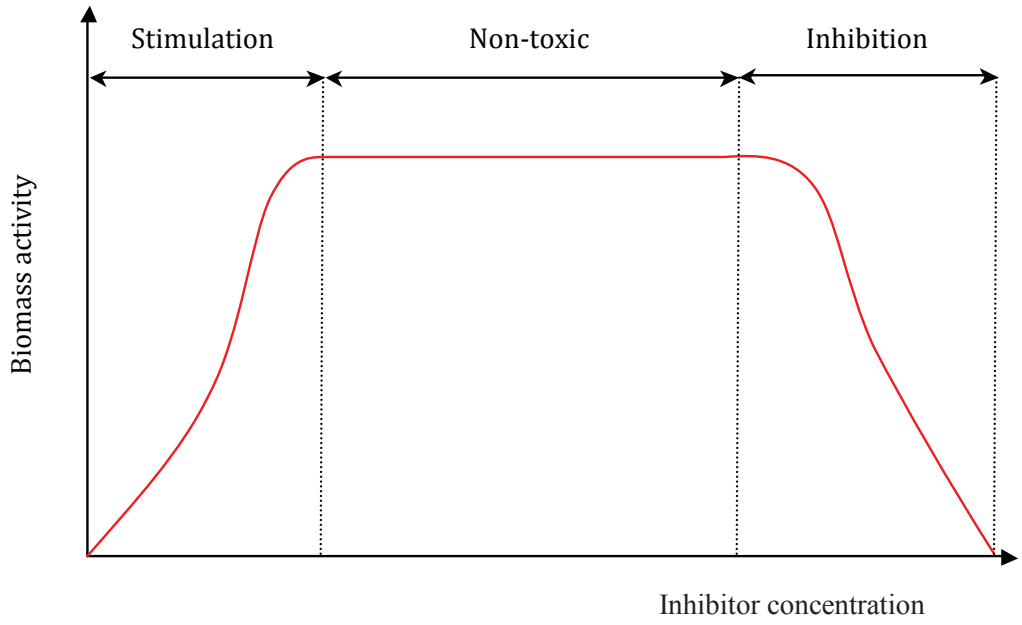

Figure 2.3. Schematic representation of the inhibition phenomenon (adapted from Angelidaki *et al.* 2006).

Ammonia and ammonium are found in anaerobic digesters as products of the biological degradation of nitrogenous matter. The unionised form (NH_3) is considered more toxic than ammonium (NH_4^+), as it is freely membrane-permeable (Mata-Alvarez *et al.* 2000; Chen *et al.* 2008). The concentration of ammonia is dependent on pH, as the concentration of hydrogen ions determines the form that will be dominant

in the system. As ammonia is also found in gaseous form, temperature affects its concentration because of its impact on gas solubility coefficients. It is believed that low levels of ammonia are beneficial to anaerobic digestion because nitrogen is a necessary nutrient for microorganisms. However, at high concentrations, ammonia inhibits microbial growth and methanogens are particularly susceptible to its presence (Kiely *et al.* 1997; Bouallagui *et al.* 2009; Ramirez *et al.* 2009).

The degradation of sulphur-rich substrates can result in inhibitory concentrations of sulphides in an anaerobic digester. Two stages are usually considered with the inhibitory effect of sulphides. The primary stage consists of the competition between sulphate-reducing bacteria and methanogens for carbonaceous substrates, which will impact both methane production and the concentration of sulphide in the system (Chen *et al.* 2008). The outcome of this competition will in turn determine the second stage of inhibition characterised by sulphide toxicity towards most microorganisms (Batstone *et al.* 2002). Sulphide in the associated form of hydrogen sulphide (H_2S) is the most toxic form to the microbial consortium and is also a gas phase component (Speece 1996).

Light metal salts are commonly found in anaerobic digesters through sodium, calcium, potassium and magnesium. They exist in anaerobic digestion systems as a result of either the breakdown of organic matter or being artificially added for pH control (Chen *et al.* 2008). Traces of these light metals are required in anaerobic systems to stimulate bacterial growth and to ensure process optimisation (Mata-Alvarez 2003), but high levels can cause serious microbial inhibition (Feijoo *et al.* 1995). The accumulation of salts negatively impact microorganisms, because of the excessive increase of the osmotic pressure regulating the water flow across the cell

membrane, which can lead to cell death (Ollivier *et al.* 1994). Inhibitory levels are dependent on adaptation of the bacterial consortium and the synergistic effects resulting from the presence of other cations (Appels *et al.* 2008; Bashir and Matin 2004a; Bashir and Matin 2004b). Similarly, heavy metals such as chromium, cobalt, iron, zinc or nickel can be found in relevant concentrations in some substrates. Their toxic effect is attributed to the disruption of enzyme function and structure (Chen *et al.* 2008).

Phenolic compounds can be grouped with long chain fatty acids under the category of potentially inhibitory organic substances. They are inhibitory to microorganisms through their interaction with cell membrane inducing leakage of intracellular constituents (McDonnell and Russell 1999; McDonnell 2007). The mechanisms of inhibition for high molecular weight phenols can be explained through the inhibition of extracellular microbial enzymes, the interference with microbial metabolism or the deprivation of the substrates necessary for microbial development (Scalbert 1991). Other potentially toxic organics include halogenated benzenes, chlorophenols and N-substituted aromatics.

2.3.3. Temperature

Temperature is a major parameter influencing the rate of anaerobic digestion since variations in temperature have an impact on thermodynamic reactions such as gas transfer rates, but also on hydrolysis and methanogenesis kinetic rates (Veeken and Hamelers 1999). Different species of methanogens thrive at three main temperature ranges: psychrophilic, mesophilic and thermophilic, but anaerobic systems are usually operated at mesophilic (~35°C) or thermophilic temperatures (~55°C) (Abbasi *et al.*

2012). Figure 2.4 shows the temperature ranges and the corresponding rates of anaerobic biodegradation.

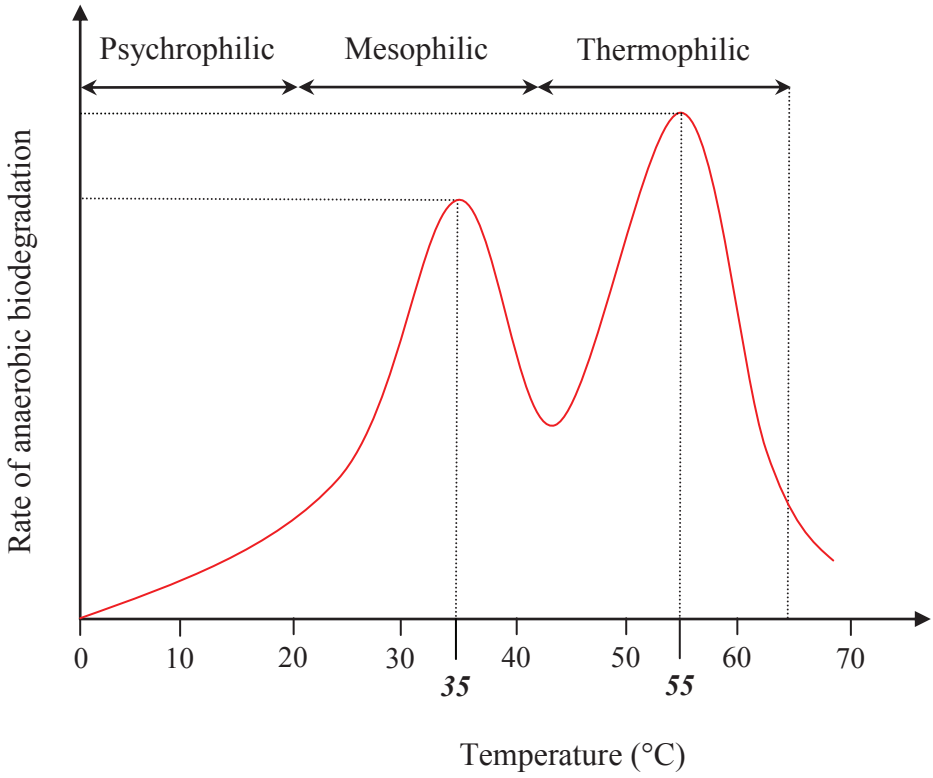

Figure 2.4. Temperature ranges for anaerobic digestion (adapted from Mata-Alvarez 2003).

The decrease in process rates corresponding to the increase of temperature from mesophilic to thermophilic conditions is associated with the negative impact of sudden temperature changes. It is thought that even small changes in temperature significantly reduce the biogas production rate (Ward *et al.* 2008). Converti *et al.* (1999) observed that the increase of temperature from mesophilic to thermophilic conditions was responsible for a decrease of methane production, but enhanced the methane content of biogas. A progressive change in temperature is likely to prevent strong changes in process rates as the microbial consortium is capable of adaptation.

Anaerobic digesters operated at mesophilic temperatures are usually favoured because of their stability and fewer energy requirements (Mata-Alvarez 2003). On the other hand, thermophilic systems exhibit better yields than mesophilic systems with increased pathogen kill-off during process and the resulting solids are often suitable for direct spreading on land (De Baere 2000; Killilea *et al.* 2000). However, thermophilic reactors are generally considered to have a lower energy balance when compared to mesophilic systems, because of the extra energy input that is required (Abbasi *et al.* 2012). The type of substrate, the hydraulic retention time and the system design also need to be considered when selecting the temperature range at which the process will take place.

2.4. Process variations

Anaerobic reactors are typically characterised into different types according to their feeding mode (continuous, semi-continuous, batch) and moisture content ('wet', 'dry' and 'semi-dry'), with large single- and two-stage reactors usually continuously fed. A further distinction can be made between the setting of reactors (horizontal or vertical) and the temperature at which they are operated. The most common types of anaerobic systems are discussed in the following sections.

2.4.1. 'Wet' and 'Dry' digestion

The total solids value is the parameter used to classify 'wet' or 'dry' digestion systems. It is thought that reactors with a total solids value of about 16% or less are 'wet' digesters, whilst 'dry' systems have between 22% and 40% total solids and those falling in between the two categories are considered 'semi-dry' (Ward *et al.*

2008). Factors such as substrate dilution, dewatering or moisture content of the feedstock define the 'wet' or 'dry' digestion as they impact directly on the total solids content, which in turn greatly affects the cost, performance and reliability of the digestion process (Vandevivere *et al.* 2003). Because of their higher water content, 'wet' systems are usually found in the form of classical completely mixed reactors with common pumping and mixing devices. The requirements of dilution water, large reactor size, and the energy required for the pumping, mixing and heating of considerable volumes of liquid are however, disadvantages of these systems (Jagadabhi *et al.* 2011). On the other hand, 'dry' systems are characterised by small water usage, small reactors with important loading rates and small heat requirements, but offer only a low possibility to dilute inhibitors. The differences between 'wet' and 'dry' systems are minimal in terms of investment and operational costs, but are considerable on environmental issues since 'wet' systems require large quantities of dilution water (Vandevivere *et al.* 2003).

2.4.2. One-stage systems

In one-stage systems, all the reactions involved during anaerobic digestion take place simultaneously in a single reactor. These systems are usually favoured over multi-stage design because their simpler layout involves smaller investment and maintenance costs with biological performances similar to more complex systems as long as the digester is well operated (Weiland 1992). A major drawback with one-stage systems is that operational conditions need to be a compromise suitable for all the biochemical processes despite the different growth rates and optimal environmental factors characterising each microbial group. The concept that optimal

conditions for both acidogenic and methanogenic microorganisms can be provided in a single reactor is therefore not achievable (Kivaisi and Mtila 1998). This disadvantage is emphasised with substrates that are easily hydrolysed and exhibit rapid acidification, thus leading to the accumulation of volatile fatty acids and the consequent inhibition of methanogenesis.

2.4.3. Two-stage systems

Two- or multi-stage systems involve the separation of biochemical steps into at least two linked reactors. Typically, the first stage harbours hydrolysis and acidification reactions whilst acetogenesis and methanogenesis occur in the second stage. With the separation of the different biodegradations steps, it then becomes possible to increase the rate of both hydrolysis and methanogenesis by applying specific operational parameters in the different reactors (Vandevivere *et al.* 2003). The simplest design of two-stage systems is two completely mixed reactors in series with technical features similar to one-stage system with most commercial designs offering a biomass retention scheme in the second stage (Vandevivere *et al.* 2003). Some authors (Gunaseelan *et al.* 1997; Azbar *et al*. 2001) differentiate two-phase and two-stage systems with the former referring to the development of different biomasses in separate reactors and the latter being relevant to the recycling of the same biomass in different environmental conditions. Figure 2.5 illustrates the conceptual differences between two-phase and two-stage systems.

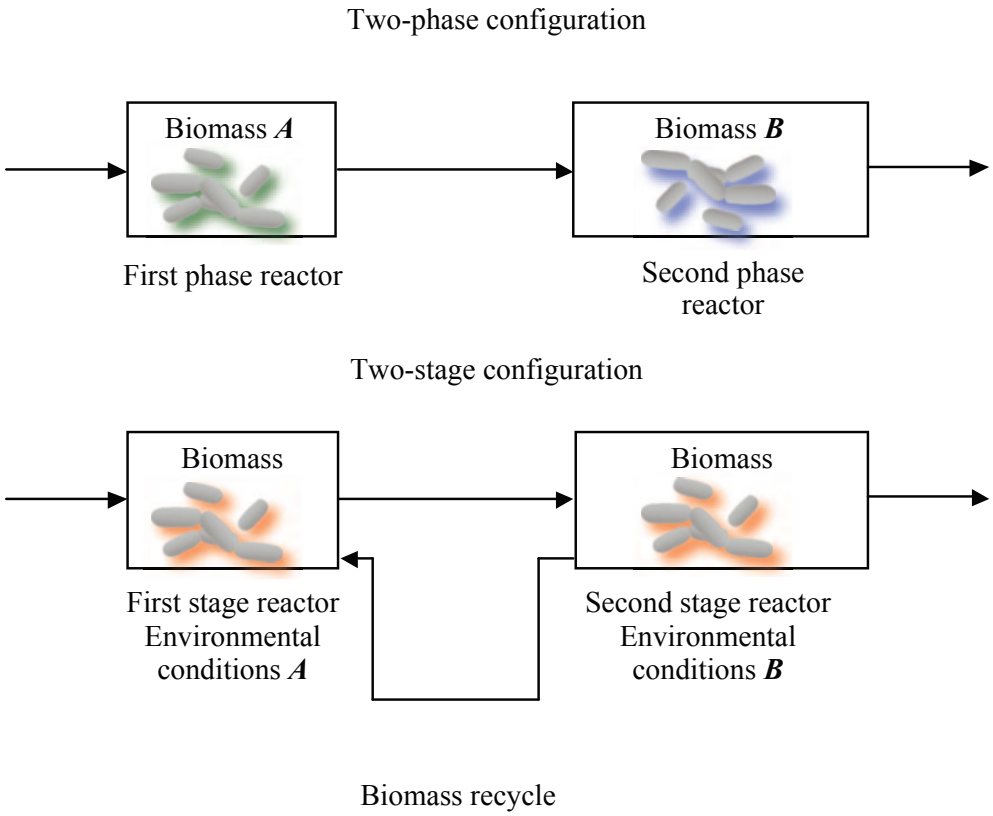

Figure 2.5. Two-phase and two-stage process configurations (adapted from Azbar *et al.* 2001).

The separation of acidogenic and methanogenic steps is mostly seen as beneficial in terms of reactor stability and biogas yield (Sosnowski *et al.* 2003), but the increased technical complexity of multi-stage systems does not always convert to higher process rates and subsequent yields (Weiland 1992).

2.4.4. Batch systems

Batch systems are the simpler anaerobic systems in which digesters are filled once with fresh substrate and allowed to go sequentially through all the biodegradation steps at a total solids value of about 30-40% (Vandevivere *et al.* 2003). In anaerobic

reactors operated in batch mode, the biogas production rate increases over time until it reaches a maximum after which most of the liquid fraction is removed. Only a small amount of digestate is left for the inoculation of the next batch (Deutsche Gesellschaft für Sonnenenergie 2005). At laboratory scale, batch systems are mostly used to determine the maximum biomethane potential of different substrates, whilst continuous-flow reactors are designed to emulate the conditions of large-scale systems and study their performance over time (Labatut *et al.* 2011). However, on a larger-scale, major drawbacks such as the need for bulking agent, the small organic loading rate or the substantial land area required have prevented the use of batch systems despite the reliability and low-costs characterising these reactors (Vandevivere *et al.* 2003).

Chapter 3
Anaerobic digestion of brown seaweed

This section introduces the general characteristics of seaweeds and the main factors affecting the anaerobic digestion of brown algae. This includes a short review of potential inhibitors present in brown seaweed species.

3.1. Classification and characteristics of seaweeds

Seaweed is the generic term for any multicellular algae that are found in the marine environment. The most common groups of seaweed are red algae (*Rhodophyta*), green algae (*Chlorophyta*) and brown algae (*Phaeophyta*) in which the dominance of the xanthophyll pigment, fucoxanthin, is responsible for its brown colour (Gupta and Abu-Ghannam 2011). All three types can be found along coastlines where stable environmental conditions predominate. Seaweeds are used as food in most oriental countries and are cultured on a considerable scale in the East when compared to Europe (Ross *et al.* 2008). Japan, South Korea and China are the largest consumers of seaweed, with the latter harvesting an estimated 5 millions wet tonnes each year (McHugh 2003). Seaweeds are also harvested for the extraction of polysaccharides, hydrocolloids or phycocolloids. Brown and red macroalgae are the main sources of commercial polysaccharides (McHugh 2003). In temperate seas, brown seaweeds commonly dominate the flora, but the pattern of species distribution differs among geographical regions, with *Ascophyllum nodosum* and *Laminaria* species being the

most common types found on the British coastline (Kelly and Dworjanyn 2008). Because of their large biomass and their high biodegradability, *Phaeophyta* are of prime interest for bioconversion to biofuels. These brown seaweed or kelps are usually found in the sublittoral zone but can grow at up to 50 meters depths (Hanssen *et al.* 1987). Figure 3.1 shows the vertical distribution at which different types of seaweed can be found on a typical European shore.

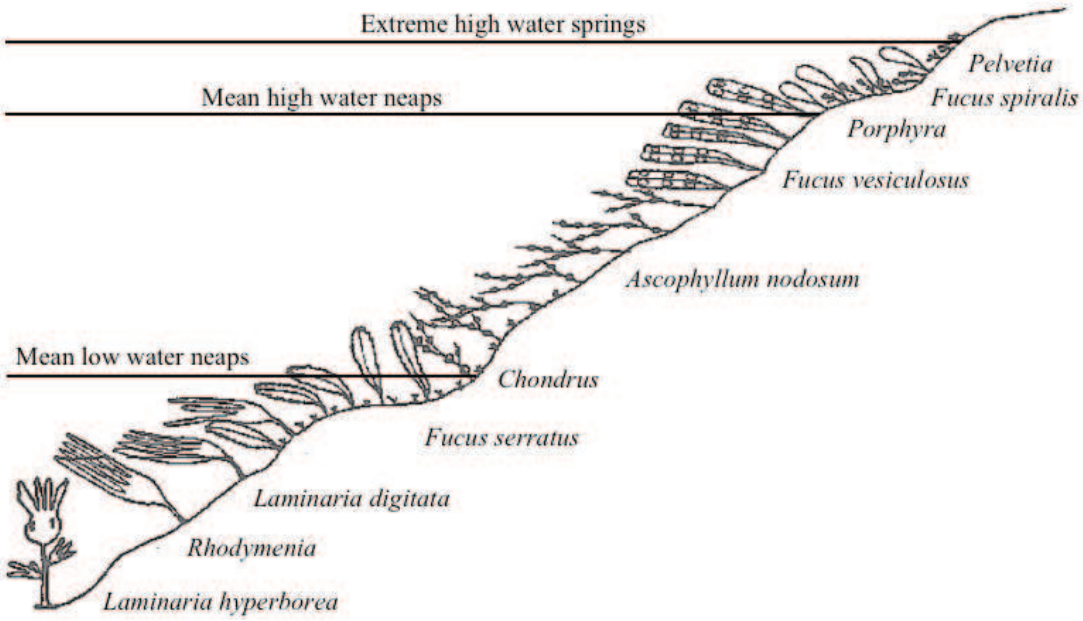

Figure 3.1. Typical zonation of different seaweed species (adapted from Chapman 1950).

With about 1800 different species, the taxa to which brown seaweeds belong to differ mainly with geographical area and in the North Atlantic, brown seaweeds principally belong to the genus *Laminaria,* whereas in Pacific temperate waters, they are often from the genus *Macrocystis* (Kelly and Dworjanyn 2008). *Laminaria* species found in the United Kingdom are usually up to 3 metres in length, and the majority of kelps in commercially harvestable densities are found in Scotland, with an estimated total of 10 million tons of seaweed that could be potentially harvested (Chapman 1948,

Woodward 1951). Seaweeds produce unique chemicals to resist the stress induced by the marine environment (Bruton *et al.* 2009). Kelps are characterised by a large flattened blade or frond, a perennial stem or stipe and a large multi-branched holdfast attached at or below the low water line (Hayward *et al.* 1996). Figure 3.2 shows illustrations of *Laminaria digitata*.

Figure 3.2. Illustrations of *Laminaria digitata* (adapted from Chapman 1950; Belsher 1986).

Because seaweeds are almost always totally immersed in water, internal transport of nutrients or water is not necessary, thus increasing their biomass productivity which has been found in the range of 3,300 to 11,300 grams dry weight.m^{-2}.$year^{-1}$ for large brown algae (Gao and McKinley 1994).

3.2. Physiology and chemical composition of brown seaweed

On a percentage basis, water is the main constituent of brown seaweeds, but their chemical composition is seasonal and varies greatly geographically and between species. On a dry basis, organic matter represents between 62-78% and 22-37% are minerals (Bruton *et al.* 2009). Organic constituents are dependent on the growth cycle of the seaweed and carbohydrate levels (mainly in the form of alginic acid, mannitol and laminaran) are usually low in winter and spring, whereas the tendency reverses during summer and autumn (Adams *et al*. 2011a). Environmental factors such as water temperature and salinity impact on the chemical composition of kelps (Marinho-Soriano *et al.* 2006), but most studies have focused on the effects of seasonal variability (Sánchez-Machado *et al*. 2004). Black (1950b) found that the cellulose content of brown seaweed species was not only dependent on the season, but also the depth of immersion at which the seaweeds were collected, hence emphasising the importance of environmental conditions. Table 3.1 shows the typical variations of elemental composition of the brown seaweed *Laminaria digitata* collected on British coasts. It can be seen that ash and nitrogen levels fluctuate during the year, whereas elemental sulphur concentrations are stable with values typically 4 times lower for brown seaweed when compared to red seaweed (Rupérez 2002).

Table 3.1. Seasonal variation in the elemental composition of *Laminaria digitata.*

Time of collection	Ash (%)	Ultimate (% by weight)				
		C	H	N	O	S
January 2008[a]	27.70	29.40	4.50	2.70	34.90	0.80
February 2005[b]	26.06	31.59	4.85	0.90	34.16	2.44
February 2008[a]	29.50	28.80	4.40	3.00	33.40	0.80
March 2008[a]	34.80	26.40	4.00	3.40	30.50	0.90
April 2008[a]	32.40	27.30	4.10	3.50	32.00	0.90
May 2008[a]	33.20	27.50	4.20	3.30	30.80	0.90
June 2008[a]	22.50	32.20	5.30	1.70	37.60	0.80
July 2008[a]	13.80	36.20	5.60	1.30	42.50	0.60
August 2008[a]	16.50	35.10	5.50	1.10	41.20	0.70
September 2008[a]	19.00	33.80	5.30	1.40	39.70	0.80
October 2008[a]	21.10	33.30	5.10	1.70	37.80	0.90
October 2010[c]	20.49	33.55	5.09	2.54	37.00	1.34
October 2011[c]	20.49	31.63	4.89	2.52	39.15	1.32
November 2008[a]	22.40	32.50	5.00	1.90	37.30	0.90
December 2008[a]	23.70	31.70	4.90	2.10	36.80	0.90

[a]Adapted from Adams *et al*. (2011a); [b]Ross *et al.* (2008) ; [c]This study

Similarly, light metal salts such as potassium, calcium, magnesium or sodium have also been observed to fluctuate during the year (Carpentier *et al*. 1988). Among the organic constituents of kelp, and according to their elemental composition, laminaran and mannitol are considered to have the highest biogas potential during anaerobic digestion (Adams *et al.* 2011b). These authors also found that the main carbohydrate fraction in the form of alginic acid had a relatively low methane yield during its anaerobic degradation.

3.2.1. Alginate

The term alginate describes the salts of alginic acid $(C_6H_8O_6)_n$, principally found in the cell walls of brown seaweed species, which are partly responsible for their flexibility (McHugh 2003). Alginate is a linear polymer consisting of (1→4) β-D-mannuronic acid (M) and (1→4) α-L-guluronic acid (G) and appears to be a structure-forming component in seaweeds (Miller 1996). Figure 3.3 shows a representation of the chemical structures of monomer units forming alginate.

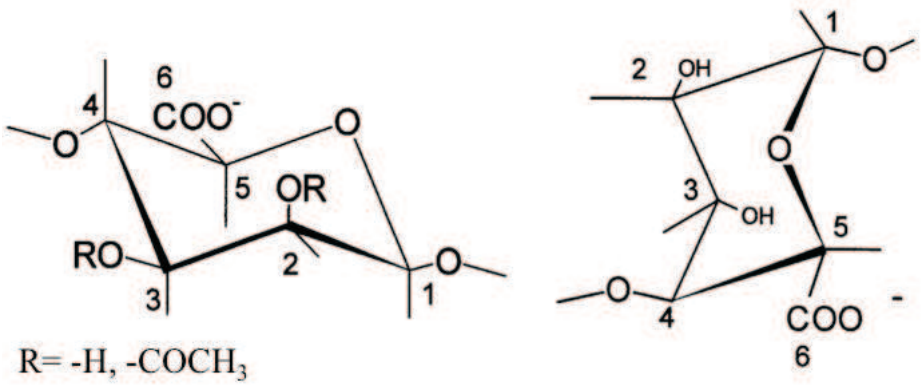

Figure 3.3. Example of chemical structures of monomer units in alginate: β-D-mannuronate (left) and α-L-guluronate (right) (adapted from Schürks *et al.* 2002).

The structure of alginate may consist of homopolymeric segments (G and M blocks) in which entire sections are composed of sequences of G or M. However, other segments may be heteropolymeric, consisting of alternating Gs and Ms (G-M-G-M-etc.) (Wingender and Winkler 1984). The exact chemical composition and sequence of G and M units depend on the biological source, growth and environmental conditions of the seaweed they have been extracted from (Gomez *et al.* 2009). In brown seaweeds, the alginate polymers accumulate and by binding divalent metals ions, such as calcium, form strong gels and give thick aqueous solutions (McHugh 2003). The anaerobic degradation of alginate has principally been reported on alginate

in solution and proved successful in both full-scale reactors and during batch assays (Carpentier *et al.* 1988; Adams *et al.* 2011b). However, alginates found in seaweed tissues are likely to be more difficult to digest, since they are organised in gels prone to protect them from enzymatic degradation (Moen and Østgaard 1997). The anaerobic degradation of alginates might be improved by depolymerisation through chemical, oxidative-reductive or enzymatical treatments (Moen 1997).

3.2.2. Mannitol and laminaran

Mannitol and laminaran are storage carbohydrates found in brown seaweeds at smaller concentrations than alginate. Mannitol ($C_6H_8(OH)_6$) is a sugar alcohol that usually constitutes between 3% to 21% of dried seaweeds (Black 1950a). Theoretical and experimental methane yields from the anaerobic degradation of mannitol are relatively high with respectively 400 and 291 cm^3 of methane generated per gram of volatile solid (Adams *et al.* 2011b). Methane yields from different seaweed species have also been found to be strongly dependent on the concentrations of mannitol (Bird *et al.* 1990). Mannitol is a soluble and readily usable carbohydrate, but it has been reported that many microorganisms are not able to carry out its degradation under strictly anaerobic conditions (Van Dijken and Scheffers 1986).

Laminaran is composed of (1→3) β-D-glucan with (1→6) β-branching, but its structure and composition vary between algal species (Rioux *et al.* 2007). Figure 3.4 shows the chemical structure of laminaran. It can be found in either soluble or insoluble forms with its solubility depending of the presence of branching and generally, the higher the branching content, the higher the solubility in cold water (Rioux *et al.* 2010). Two types of laminaran have been reported, one type with chains

that are terminated by D-mannitol residues (M-series) and another type with chains terminated by D-glucose residues (G-series) (Nelson and Lewis 1974).

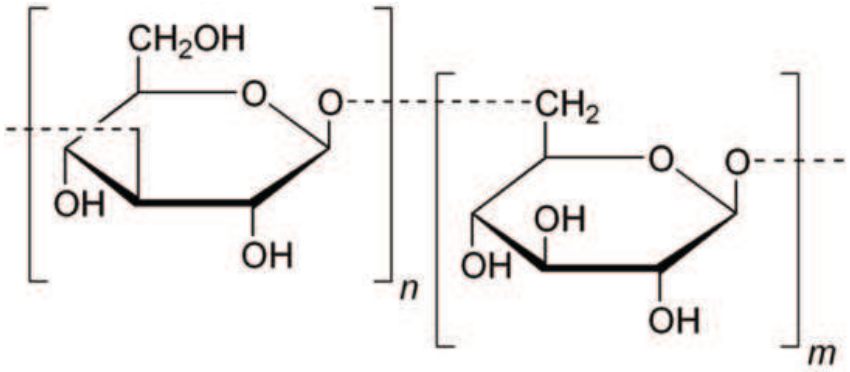

Figure 3.4. Chemical structure of laminaran: (1→3) β-D-glucan (left) with (1→6) β-branching (right).

Many microorganisms can hydrolyse laminaran to its glucose monomer and it seems to be readily degradable under anaerobic conditions (Troiano *et al.* 1976; Adams *et al.* 2011b). During the anaerobic digestion of seaweed, laminaran is considered as a minor organic component (Gunaseelan 1997).

3.2.3. Other organic compounds

Algal fucoidan is a sulphated fucan mainly composed of fucose, uronic acids, galactose, xylose and sulphated fucose for which the exact structural characteristics have not yet been elucidated (Rioux *et al.* 2007). Fucoidans are water-soluble and demonstrate a variety of pharmaceutically relevant biological applications through their anticoagulant, antithrombotic and antiangiogenic activities (Hahn *et al.* 2012). The structural studies of this polysaccharide have somehow been limited because the extraction and isolation of fucoidans in a pure form is relatively complex (Marais and

Joseleau 2001), but its enzymatic degradation has been found possible (Daniel *et al.* 1999). The protein content of brown seaweed is relatively low, when compared to green and red seaweeds, with proteins representing about 3% to 15% of the seaweed dry weight (Fleurence 1999). However, algal proteins have been reported as having a low digestibility, presumably due to their binding by polyphenolic compounds found in kelps (Moen *et al.* 1997). For most seaweeds, aspartic and glutamic acids together form the largest part of the amino acid fraction (Fleurence 1999).

3.3. Factors affecting the anaerobic biodegradation of brown seaweed

3.3.1. Cellulose and lignin

Cellulose is readily biodegradable under anaerobic conditions, but tends to become less degradable and even completely refractory to biodegradation when incorporated into the lignocellulose complex (Sanders *et al.* 2003). If lignin is most commonly found in the cell wall of woody biomass, its late discovery in red seaweed (Martone *et al.* 2009) has raised interest on the probable occurrence of lignin-like structures that could impair the anaerobic degradation of other types of seaweeds. Gómez-Ordóñez *et al.* (2010) found that about 10% of the insoluble fibres found in brown seaweed contained lignin, with the remaining being mainly cellulose. Most algal cells are surrounded by a polysaccharides-rich cell wall, such as cellulose, that might be recalcitrant to microbial degradation (Stengel *et al.* 2011). The cellulose content of brown seaweed has been found to be dependent upon environmental conditions, since cellulose reinforces the structure formed by alginic acid with its concentration thus being dependent on the seaweed strength requirements and hence, the depth of immersion (Black 1950b).

3.3.2. Salts

Light metals ions are found at various concentrations in brown seaweed with the most common being sodium, calcium, potassium, magnesium, barium and strontium (Adams *et al.* 2011a). They are absorbed by seaweed from the surrounding seawater being naturally characterised by high concentrations of light metal salts. In the perspective of the anaerobic degradation of seaweed, high salinity levels mainly results from the fact that raw seaweed contains large amounts of seawater that are ultimately introduced into the anaerobic digestion system. Traces of sodium, calcium, potassium and magnesium are required in anaerobic systems to stimulate bacterial growth (Mata-Alvarez 2003; Appels *et al.* 2008), but high levels are expected to cause microbial inhibition (Feijoo *et al.* 1995). Particularly, the high sodium concentration during the anaerobic treatment of marine biomass is a serious issue (Aspé *et al.* 1997; Jeison *et al.* 2008; Sialve *et al*. 2009). Potassium, calcium and magnesium ions have also been reported inhibitory to non-acclimatised microorganisms (Fang *et al.* 2011).

Some authors have reported relatively high accumulation of propionate and acetate in anaerobic systems receiving saline feedstock, indicating that high salinity levels may have a greater impact on the acetogenic bacteria and acetoclastic methanogens than on acidogens (Rinzema *et al.* 1987; Liu and Boone 1991; Kimata-Kino *et al.* 2011). The methanogenic archaea found in anaerobic reactors are particularly sensitive to sodium concentrations and methanogenesis has been reported to be strongly inhibited at sodium levels exceeding 10 g/l (Lefebvre and Moletta 2006). Different inhibitory concentrations of sodium have been reported in the literature, also suggesting that the level of microbial sodium inhibition may be dependent on many factors, such as system design, operation and seed inoculum. Soto *et al.* (1993) reported that a sodium ion concentration ranging between 14 to 18 g/l

can reduce methanogenic activity by up to 50% (IC_{50}). With granular sludge, Rinzema *et al.* (1987) found an IC_{50} value of about 10 g Na^+/l whilst Feijoo *et al.* (1995) reported an IC_{50} of 16.3 g Na^+/l with saline-adapted seed inoculum. The use of an anaerobic reactor sludge bed fitted with a microfiltration membrane can increase the IC_{50} to an even higher value of 25 g Na^+/l (Jeison *et al.* 2008).

Sodium affects oxidation mechanisms and is involved in the transportation of substrate and ions through the cell membrane (Fang *et al.* 2011). At high levels, sodium causes cell plasmolysis, which impacts on microorganisms through the variation of osmotic pressure (Lefebvre and Moletta 2006; Kapdan and Erten 2007). An excessive increase in the osmotic pressure regulating the water flow across the cell membrane hence results in cell death (Ollivier *et al.* 1994). Methanogens are severely affected by sodium toxicity and at high salt concentrations, cells consume a significant amount of energy to adapt to the osmotic environment and hence produce less methane (Vyrides and Stuckey 2009). Strategies to reduce the negative impact of high sodium concentrations on microorganisms have been focused on the use of salt-tolerant microorganisms or the adaptation of methanogens to gradually increasing sodium levels (Chen *et al.* 2008). It has also been observed that the inhibition caused by sodium tends to be reduced with the addition of other cations such as potassium or calcium (Bashir and Matin 2004a).

3.3.3. Phenolic compounds

Tannins are phenolic compounds classified in three main classes, namely hydrolysable tannins, condensed tannins and phlorotannins with the latest being exclusively found in brown seaweed (Jormalainen and Honkanen 2008). Stored within cells in vessels called physodes, phlorotannins have been reported from almost

all brown algae species and have been proposed to play ecological roles in wound healing, microbial infection, metal ion chelation or UV protection even if they can have primary roles in algal cell wall biosynthesis (Maschek and Baker 2008).

Phlorotannins are polymers or oligomers of phloroglucinol (1,3,5-trihydroxybenzene) and are classified into various groups based on their chemical structure (Amsler and Fairhead 2006). Although terrestrial and marine polyphenols are similar in some aspects, there are fundamentals differences regarding their chemical structure (Shibata *et al.* 2002). Figure 3.5 shows a representation of the structures of phloroglucinol and polymeric derivates. Fucols are formed when benzene aromatic rings are exclusively connected by aryl-aryl bonds; fuhalols are made from phloroglucinol units connected with ether bridges containing an extra OH-group in every third ring; and eckols are formed when there is at least one three-ring moiety with a dibenzodioxin element substituted by a phenol group (Koivikko 2008). The molecular weights of phlorotannins can vary between 126 Daltons (Da) to 650 kDa but are usually found between 10 and 100 kDa (Boettcher and Targett 1993). The formation of phlorotannins occurs through the polyketide pathway (Arnold and Targett 2002), but the exact biosynthetic pathway and potential methodologies to monitor phlorotannin synthesis still remain unknown (Koivikko 2008).

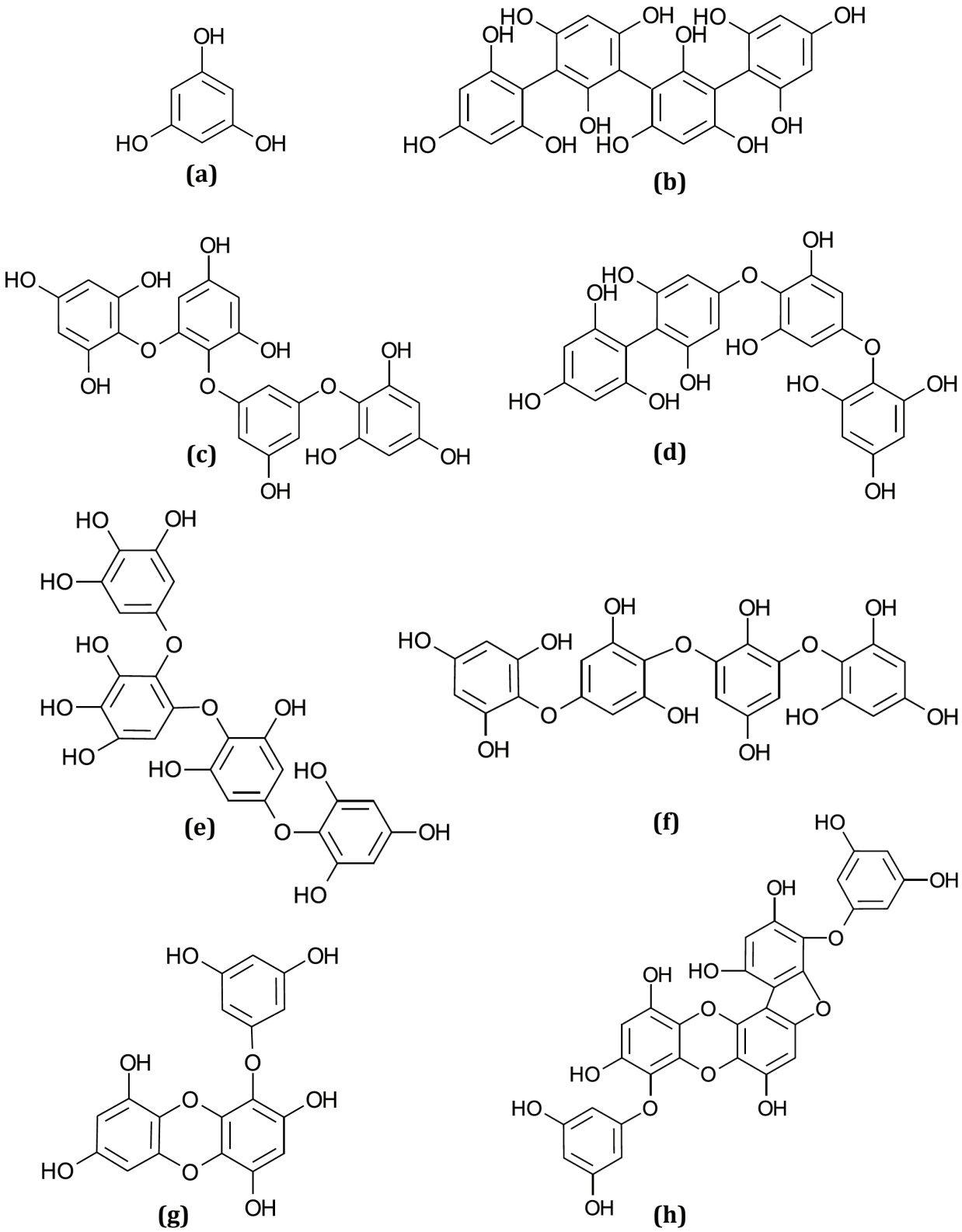

Figure 3.5. Structures of phloroglucinol (a), tetrafucol A (b), tetraphloretol B (c), fucodiphlorethol A (d), tetrafuhalol A (e), tetraisofuhalol (f), eckol (g), and phlorofucofuroeckol (h).

The occurrence of phlorotannins in brown seaweed has been linked with their defence against predation (Haavisto *et al.* 2010) and they can account for up to 20% of the seaweed dry weight (Ragan and Glombitza 1986; Amsler and Fairhead 2006). However, the role of phlorotannins as defence metabolites is not straightforward and their concentrations vary over spatial and temporal scales with their effect depending on both algae and predator species (Haavisto *et al.* 2010).

Phlorotannins have been found to have strong antimicrobial activity and recent studies have emphasised their potential as a possible new source of natural antimicrobial agents for the pharmaceutical and food industries (Eom *et al.* 2012). Dubber and Harder (2008) found antibacterial effects of algal metabolites at, and even below, algal tissue level concentration from extracts of *Laminaria digitata*. Nagayama *et al.* (2002) also reported that crude phlorotannins extracted from the brown seaweed *Ecklonia kurome* showed an increase in bactericidal activities against pathogenic bacteria. In anaerobic systems, decreased methanogenic performances have been observed by Moen *et al.* (1997) during the digestion of *Ascophyllum nodosum*. By analogy with tannins from terrestrial sources, possible mechanisms to explain antimicrobial activity of phlorotannins include, (i) inhibition of extracellular microbial enzymes, (ii) inhibition of microbial metabolism or (iii) deprivation of the substrates necessary for microbial development (Scalbert 1991). Dubber and Harder (2008) have also suggested that chemical defences of marine macroalgae are influenced by both environmental and biological factors thus influencing the levels of phlorotannin and other secondary metabolites. Through their antimicrobial activity and relatively high concentrations found in brown seaweeds, phlorotannins are thus likely to impact on the anaerobic degradation of kelps.

3.3.4. Other potential inhibitors

Additional inhibitors relevant to the anaerobic digestion of seaweed can be ammonia nitrogen, hydrogen sulphide or acrylic acid. Relatively high nitrogen concentrations in seaweed could lead to a substantial release of free ammonia (NH_3) and this is toxic to anaerobic microorganisms as mentioned in section 2.3.2. The formation of hydrogen sulphide at inhibitory levels needs to be taken into account, even if it has been mainly reported during the anaerobic digestion of green and red algae (Cecchi *et al.* 1996; Peu *et al.* 2011). Seawater also naturally contains high sulphate concentrations. Sulphur has not yet been reported as problematic during the digestion of brown seaweed (Kelly and Dworjanyn 2008) and only low values of elemental sulphur are usually found for *Laminaria digitata* as can be seen in Table 3.1. Sulphate reducing bacteria (SRBs) are usually favoured to methanogens at chemical oxygen demand to sulphate ion ratios below five and should be monitored (Briones *et al.* 2007). Acrylic acid ($C_3H_4O_2$) found in seawater is a product of dimethyl sulphide cleavage and inhibits the growth of most of bacterial species from low to medium concentrations (0.012-12 mg/ml) (Van Alstyne 2008). However, acrylic acid has not been reported so far in brown seaweed and the concentrations found in seawater are presumably too low to inhibit microorganisms (Slezak *et al.* 1994).

Chapter 4
Modelling of anaerobic digestion

This chapter provides an overview of the mathematical modelling of anaerobic digestion. It also explores the structure of the Anaerobic Digestion Model No.1, which has been adopted in this study as a platform for process simulation.

4.1. Generic mathematical process models

Attempts to model complex biochemical processes including anaerobic digestion mechanisms is not new. The mathematical description of real processes finds various applications ranging from the prediction and control of process behaviour to the testing of hypotheses that cannot be verified experimentally. Mathematical models are also used to help in our understanding of complex systems or during design and operation phases. Generally, a model transforms a set of inputs into at least one output of interest by using an established relationship, which will define the structure and the complexity of the model. These relationships are commonly a set of mathematical equations with varying complexity that correlate outputs to inputs. Equations are structured around variables that may change dynamically during simulation and parameters that are commonly used to describe objects statically and can be estimated from an *a priori* knowledge or existing data. Models characterised by constantly changing variables are referred to as dynamic in contrast with steady-state models where variables are static. Models can further be separated into linear and non-linear

categories, depending upon whether their outputs are directly proportional to their inputs and can be evaluated through the differentiation of the model equations. Linearity is particularly important during the solving of equations, since for linear models, simple analytical solutions can be obtained while for non-linear models, complex solving numerical techniques are required. Due to the intrinsic complexity of biochemical phenomena, the more elaborate models used in anaerobic digestion are principally dynamic and non-linear.

Graef and Andrews (1974), were among the first group of researchers to model anaerobic digestion. They considered the conversion of volatile fatty acids to methane through acetoclastic methanogenesis as the rate-limiting step. Subsequent models considered hydrolysis or acetogenesis prior to methanogenesis (Hill and Barth 1977; Kleinstreuer and Poweigha 1982; Moletta *et al.* 1986) using Monod kinetics (Monod 1949) with substrate inhibition as defined in Equation 4.1 (Andrews 1969):

$$\mu = \frac{\mu_{max}}{1 + \frac{K_S}{S} + \frac{I}{K_I}} \qquad \textbf{(4.1)}$$

Where, μ is the specific microbial growth rate, μ_{max} is the maximum specific growth rate, K_S is the half-saturation constant, S is the concentration of growth-limiting substrate, K_I is the inhibition constant and I is the inhibitor concentration. This equation is based on the kinetics of the bacterial activity considering that when cells of specific microorganisms are put into contact with an excess of appropriate substrate in suitable environmental conditions, microbial growth occurs (Garcia-Heras 2003). However, this microbial growth μ can be restrained by a limiting concentration of substrate or any inhibitory substance. If these models were mostly appropriate to

describe anaerobic digester behaviour at steady-state, they would not be reliable under transient operating conditions and the implementation of the key physico-chemical and biochemical processes, along with the combined effect of several inhibitors, was necessary to accurately describe the digestion process (Lyberatos and Skiadas 1999). Subsequent models mostly used kinetic equations to model bacterial activity (Linke 2006) and focused on hydrolysis, due to its being the rate-limiting step for most substrates (Christ *et al.* 2000; Sanders *et al.* 2000). Models were also built to consider other biochemical processes occurring during anaerobic degradation (Kiely *et al.* 1997; Batstone *et al.* 2000; Liu *et al.* 2008) and sometimes based on different kinetic expressions for microbial growth such as defined by Contois (1959) to take into account mass transfer limitations leading to the variation of μ with microbial population density as shown on Equation 4.2:

$$\mu=\mu_{max}\frac{S}{K\cdot X+S} \qquad \textbf{(4.2)}$$

Where, μ is the specific microbial growth rate, μ_{max} is the maximum specific growth rate, K is the specific substrate utilisation rate coefficient, X is the biomass concentration and S is the concentration of growth-limiting substrate. The equation can also be corrected with inhibition factors, such as for Equation 4.1. Chen and Hashimoto (1978) updated the formula used by Contois to include the influence of the initial concentration of substrate and express mass transfer limitation. Table 4.1 shows a non-exhaustive list of models used in anaerobic digestion and the processes they take into account.

Table 4.1. Anaerobic digestion models (adapted from Lyberatos and Skiadas 1999).

Model	Processes	Kinetic function	Inhibition
Graef and Andrews (1974)	Methanogenesis	Andrews	VFA, external inhibitor
Hill and Barth (1977)	Hydrolysis, acidogenesis, methanogenesis	Andrews	VFA, NH_3
Hill (1982)	Acidogenesis, acetogenesis, homoacetogenesis	Monod	VFA
Kleinstreuer and Poweigha (1982)	Acetogenesis, methanogenesis	Andrews	Acetate, toxic substances
Mosey (1983)	Acidogenesis, acetogenesis, methanogenesis	Monod	H_2 partial pressure
Bryers (1985)	Hydrolysis, acidogenesis, acetogenesis, methanogenesis	First order, Monod	/
Moletta *et al.* (1986)	Acetogenesis, methanogenesis	Andrews	Acetate
Smith *et al.* (1988)	Hydrolysis, acidogenesis, methanogenesis	First order, Andrews	VFA
Pullammanappallil *et al.* (1991)	Acidogenesis, acetogenesis, methanogenesis	Monod, Andrews	H_2 partial pressure, butyrate and propionate
Costello *et al*. (1991)	Acidogenesis, acetogenesis, methanogenesis	Monod	pH, H_2 partial pressure
Angelidaki *et al.* (1993)	Enzymatic hydrolysis, acidogenesis, acetogenesis, methanogenesis	First order, Monod	VFA, acetate, NH_3
Siegriest *et al.* (1993)	Hydrolysis, fermentation, acetogenesis, methenogenesis	First order, Monod	pH, H_2 partial pressure, NH_3
Kiely *et al.* (1997)	Hydrolysis, acidogenesis, methanogenesis	Andrews	pH, NH_3
Christ *et al.* (2000)	Hydrolysis	First order	/
Sanders *et al.* (2000)	Hydrolysis	First order	Particle size distribution
Batstone *et al.* (2000)	Enzymatic hydrolysis, acidogenesis, acetogenesis, methanogenesis	First order	pH, VFA, NH_3
Keshtkar *et al.* (2001)	Enzymatic hydrolysis, acidogenesis, acetogenesis, methanogenesis	First order, Monod	pH, VFA, acetate, NH_3
Vavilin *et al.* (2001)	Hydrolysis, acidogenesis, acetogenesis, methanogenesis	Contois, first order	pH
Liu *et al.* (2008)	Hydrolysis, acidogenesis, methanogenesis	Monod, Andrews	Acetate, NH_3
Martin Santos *et al.* (2010)	Hydrolysis, acidogenesis, acetogenesis, methanogenesis	First order	VFA

Some authors decided to limit the complexity of their model and therefore, ease the calibration step, but still obtain sufficiently accurate model predictions (Vavilin *et al.*

2001; Martin Santos *et al.* 2010) or use elaborate mathematical tools to optimise the accuracy of their models (López and Borzacconi 2010). Rodriguez *et al.* (2008) used a model structure characterisation method to determine the mathematical relationships between variables and produce a reduced model that retains most of the system variability and outputs accuracy. In that sense, parametric uncertainty and sensitivity analyses are appropriate tools to reduce the complexity of mathematical models by assessing which parameters require a careful calibration.

In the late 1990s, most researchers believed that a generic platform for the modelling of anaerobic digestion, regrouping most of the processes involved and taking into account advances made to date, was required in order to reach a common basis for further model development (Donoso-Bravo *et al.* 2011). This led to the setting up of the International Water Association (IWA) Task Group for Mathematical Modelling of Anaerobic Digestion Processes, and consequently to the development of the Anaerobic Digestion Model No.1 (ADM1) (Batstone *et al.* 2002) as a detailed reference model.

4.2. The Anaerobic Digestion Model No.1 (ADM1)

4.2.1. Introduction

The ADM1 has been successfully applied to model the anaerobic digestion of domestic wastewater (Feng *et al.* 2006), organic waste (Derbal *et al.* 2009), olive pulp and olive oil wastewater (Kalfas *et al.* 2006; Fezzani and Ben Cheikh 2008), municipal wastewater solids (Johnson and Shang 2006; Yasui *et al.* 2007), agro-wastes (Gali *et al.* 2009) and grass silage (Koch *et al.* 2010; Thamsiriroj and Murphy 2010). Since its publication, the ADM1 has seen several updates including the addition of new processes not taken into account in the original version. The model

structure has also seen extensive updates with the introduction of surface-limiting kinetics to describe the hydrolysis of complex substrates (Yasui *et al.* 2007; Zhao *et al.* 2009), the improvement of mass and charge balances calculation (De Gracia *et al.* 2006), the introduction of Contois functions for the hydrolysis of carbohydrates (Mairet *et al.* 2011) and the addition of sulphate reduction processes (Fedorovich *et al.* 2003). When reviewing the ADM1 modifications and applications over a period of four years after the publication of the original version, Batstone *et al.* (2006) found that about 30 papers have been published on the modelling of anaerobic digestion using the ADM1 as a simulation platform. The wide acceptance of the model can partly be explained by the fact that it has been recognised as an excellent tool to manage and monitor digester operation (Derbal *et al.* 2009; Gali *et al.* 2009) or for process simulation and design (Blumensaat and Keller 2005; Koutrouli *et al.* 2009).

However, some drawbacks are also associated with the ADM1. The most common criticism is the number of compromises and shortcomings found in the model structure (Batstone *et al.* 2002), with some of the latter being considered as illogical assumptions and methods (Kleerebezem and Van Loosdrecht 2006). In order to take into account most of the processes involved in anaerobic digestion, the model is characterised by a complicated structure associated with a large number of parameters and variables. This over-parameterisation has been reported to impact negatively on the model practicability (Parker 2005; Lee *et al.* 2009). Anaerobic digestion is characterised by a large range of timescales, with some processes (mainly chemical reactions) taking place within minutes and others (mainly biological activities) over months. When translated into a system of equations, the speeds at which all these processes occur produce a really stiff system that requires the use of specific mathematical solvers and powerful calculators, thereby limiting the wider use

of the model (Rosen and Jeppsson 2006; Rosen *et al.* 2006; Vrecko *et al.* 2006). The conversion processes considered in the model are shown on Figure 4.1. Disintegration of particulate material, hydrolysis, acidogenesis from sugars, amino acids and long chain fatty acids (LCFA), acetogenesis from LCFA, propionate, butyrate and valerate, acetoclastic methanogenesis and hydrogenotrophic methanogenesis are implemented in the ADM1.

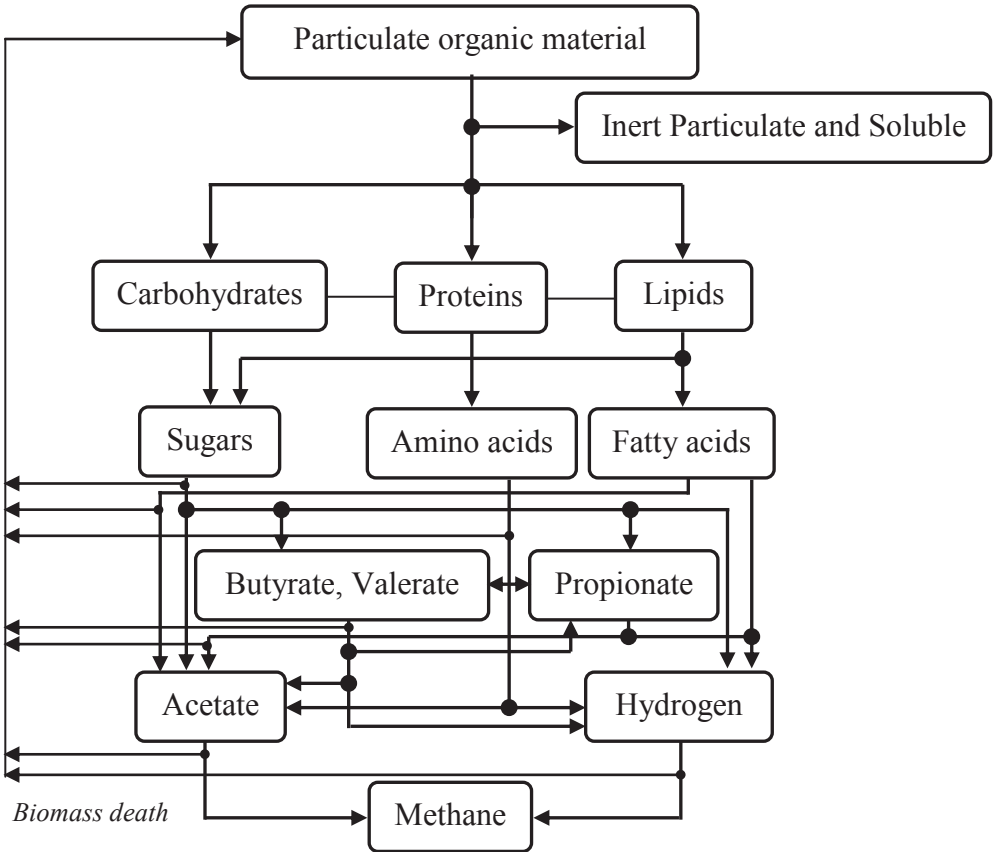

Figure 4.1. Scheme of the anaerobic degradation steps described in the ADM1 (adapted from Batstone *et al.* 2002).

The model consists of twelve differential equations representing soluble matter concentrations in the liquid phase including inorganic carbon and inorganic nitrogen, a further twelve differential equations for particulate matter concentrations, another two equations to model cations and anions levels and a final six differential equations

for acid-base reactions. Hydrogen, methane and carbon dioxide are the main components in the gaseous phase that are taken into account in the ADM1. Inhibitions that can be caused by low pH, lack of inorganic nitrogen or high levels of ammonia nitrogen or hydrogen are introduced by a set of algebraic equations. Biochemical rate coefficients and kinetic rate equations are represented by a rate matrix involving nineteen biochemical rate processes. Dead biomass is included in the complex particulate pool (Batstone *et al.* 2002). The nomenclature and modelling of major reactions and processes are further described in the subsequent parts of this chapter. Despite some limitations and an extensive number of biochemical, stoichiometric and physicochemical parameters, the ADM1 remains a platform of choice for the simulation of anaerobic digestion.

4.2.2. Nomenclature and description of parameters and variables

The ADM1 uses chemical oxygen demand (kgCOD/m^3) as the main unit for organic compounds since it is widely used in wastewater measurements and allows for a certain consistency with other IWA models. It otherwise employs a molar basis (kmol/m^3 or M) for other components such as inorganic carbon or inorganic nitrogen. Temperature, pressure, energy and time are expressed as kelvin, bar, joules and day respectively. The four main types of parameters and variables found in the ADM1 are stoichiometric coefficients, equilibrium coefficients, kinetic parameters and dynamic state/algebraic variables. Stoichiometric coefficients are used to express carbon and nitrogen contents of components, rate coefficients of components on processes, and yield of products on substrates. Equilibrium coefficients group constants used in physico-chemical processes such as acid-base coefficients, Henry's law coefficients, gas law constant (R=8.314 J/mol×K) or free energy. Kinetic parameters and rates

mainly concern biochemical processes with decay rates, Monod maximum specific uptake rates or maximum specific growth rates (see Equation 4.1) and yield of biomass on substrate. Temperature, volume, gas pressure and pH are among the variables considered in the last category of dynamic state and algebraic variables for a total of 105 kinetic and stoichiometric parameters. The ADM1 being a dynamic model, also contains dynamic state variables used in both differential and algebraic implementation. Dynamic state variables are calculated at a specific time *t* as a solution of the set of differential equations which is defined by process rates, process configuration, inputs and the model initial conditions (Batstone *et al.* 2002). Therefore the model is defined by 26 to 32 dynamic state variables depending on its implementation.

Different approaches can be found from the literature in regard to the determination of the required model parameters. Kinetic, stoichiometric and equilibrium parameters and coefficients are in most cases set to values recommended in the original model or in specific implementations reports (Batstone *et al.* 2002; Rosen and Jeppsson 2006). The non-optimisation of these parameters, particularly those associated with the metabolism and kinetic rates of microorganisms, is often reported due to experimental and calculation limitations (Wett *et al.* 2007; Dereli *et al.* 2010; Ntaikou *et al.* 2010) and it is considered that only those parameters necessary to explain the observed mechanisms should be calibrated (Donoso-Bravo *et al.* 2011). However, the input parameters corresponding to the substrate fed into the system under consideration need to be determined and often represent a major difficulty associated with the use of the ADM1 (Johnson and Shang 2006). The most straightforward approach, though rarely used, is the determination of all the input parameters by a thorough characterisation of the substrate chemical composition

(Fezzani and Ben Cheikh 2008). If the technique has the advantage of being accurate, it is time-consuming and requires the use of a wide range of analytical devices. Because the complexity of the model determines to a certain extent the number of parameters involved, Morel *et al.* (2006) used the ADM1 as a platform to develop a variable structure model that would offer a compromise between model complexity and simplicity of the parameter estimation procedure. Trial and error approaches using experimental data can also be used (Ramirez *et al.* 2009), but might be tedious and lead to ill conditioning of the parameter estimation problem when using minimisation methods (Tartakovsky *et al.* 2008). Various authors have developed specific procedures aimed at simplifying the determination of input parameters. Kleerebezem and Van Loosdrecht (2006) developed an algorithm using the elemental composition of the substrate and its general definition in the model to determine the complete input required by the ADM1. A methodology for the characterisation of the influent sludge based on the application of the mass continuity to all model transformations and elemental composition of the substrate was also developed by Huete *et al.* (2006). For specific substrates, the correlation between measured experimental parameters and the model specific inputs has been found as a suitable method to characterise the substrate in terms of carbohydrates, proteins and lipids (Lübken *et al.* 2007; Wichern *et al.* 2009). Another method consists in the transformation of a set of practical measurements usually conducted in wastewater analysis into the input vector required by the ADM1 according to stoichiometric coefficients (Zaher *et al.* 2009a).

4.2.3. Modelling of biochemical reactions

The ADM1 considers three principal biochemical steps simultaneously occurring at a cellular level. These are, acidogenesis, during which two groups of microorganisms degrade monosaccharides and amino acids to organic acids, carbon dioxide and hydrogen. Acetogenesis, where organic acids are converted to acetate, carbon dioxide and hydrogen, and methanogenesis, during which two groups of microorganisms transform acetate and hydrogen to methane during acetoclastic methanogenesis and hydrogenotrophic methanogenesis respectively, as can be seen in Figure 4.1. These transformations are characterised through their uptake, growth and decay rates. Additionally, two extra-cellular steps are included with disintegration and hydrolysis, during which large particulate organic material is reduced to shorter chain polymers such as carbohydrates, proteins and lipids later converted to monomeric compounds.

The rate of biochemical processes occurring at a cellular level is defined by substrate uptake based on Monod-type kinetics, whereas biomass decay, disintegration and hydrolysis follow first-order kinetics (Batstone *et al.* 2002). The influence of temperature is also implemented in the model original structure because of its effect on reactions rates and thermodynamics for all biochemical processes. Biochemical rate coefficients and kinetic rate equations for both soluble and particulate components are presented in a rate equation matrix shown on Table 4.2 and Table 4.3. The advantage of such a presentation is that for each component, the mass balance within a system boundary can be expressed as in Equation 4.3 (Henze *et al.* 2000):

$$\text{Accumulation} = \text{Input} - \text{Output} + \text{Reaction} \qquad \textbf{(4.3)}$$

Table 4.2. Biochemical rate coefficients and kinetic rate equations for soluble components (adapted from Batstone *et al.* 2002).

Component → i		1	2	3	4	5	6	7	8	9	10	11	12	Rate (ρ_j, kg COD.m^{-3}.d^{-1})
j	Process ↓	S_{su}	S_{aa}	S_{fa}	S_{va}	S_{bu}	S_{pro}	S_{ac}	S_{h2}	S_{ch4}	S_{IC}	S_{IN}	S_I	
1	Disintegration												$f_{sI,xc}$	$k_{dis}X_c$
2	Hydrolysis Carbohydrates	-1												$k_{hyd,ch}X_{ch}$
3	Hydrolysis of Proteins		1											$k_{hyd,pr}X_{pr}$
4	Hydrolysis of Lipids	$1-f_{fa,li}$		$f_{fa,li}$										$k_{hyd,li}X_{li}$
5	Uptake of Sugars	-1				$(1-Y_{su})\,f_{bu,su}$	$(1-Y_{su})\,f_{pro,su}$	$(1-Y_{su})\,f_{ac,su}$	$(1-Y_{su})\,f_{h2,su}$		$-\sum_{i=1-9,11-24} C_i\nu_{i,5}$	$-(Y_{su})\,N_{bac}$		$k_{m,su}\frac{S_{su}}{K_S+S}X_{su}I_1$
6	Uptake of Amino Acids		-1		$(1-Y_{aa})\,f_{va,aa}$	$(1-Y_{aa})\,f_{bu,aa}$	$(1-Y_{aa})\,f_{pro,aa}$	$(1-Y_{aa})\,f_{ac,aa}$	$(1-Y_{aa})\,f_{h2,aa}$		$-\sum_{i=1-9,11-24} C_i\nu_{i,6}$	$N_{aa}-(Y_{aa})\,N_{bac}$		$k_{m,aa}\frac{S_{aa}}{K_S+S_{aa}}X_{aa}I_1$
7	Uptake of LCFA			-1				$(1-Y_{fa})\,0.7$	$(1-Y_{fa})\,0.3$			$-(Y_{fa})\,N_{bac}$		$k_{m,fa}\frac{S_{fa}}{K_S+S_{fa}}X_{fa}I_2$
8	Uptake of Valerate				-1		$(1-Y_{c4})\,0.54$	$(1-Y_{c4})\,0.31$	$(1-Y_{c4})\,0.15$			$-(Y_{c4})\,N_{bac}$		$k_{m,c4}\frac{S_{va}}{K_S+S_{va}}X_{c4}\frac{1}{1+S_{bu}/S_{va}}I_2$
9	Uptake of Butyrate					-1		$(1-Y_{c4})\,0.8$	$(1-Y_{c4})\,0.2$			$-(Y_{c4})\,N_{bac}$		$k_{m,c4}\frac{S_{bu}}{K_S+S_{bu}}X_{c4}\frac{1}{1+S_{va}/S_{bu}}I_2$
10	Uptake of Propionate						-1	$(1-Y_{pro})\,0.57$	$(1-Y_{pro})\,0.43$		$-\sum_{i=1-9,11-24} C_i\nu_{i,10}$	$-(Y_{pro})\,N_{bac}$		$k_{m,pr}\frac{S_{pro}}{K_S+S_{pro}}X_{pro}I_2$
11	Uptake of Acetate							-1		$(1-Y_{ac})$	$-\sum_{i=1-9,11-24} C_i\nu_{i,11}$	$-(Y_{ac})\,N_{bac}$		$k_{m,ac}\frac{S_{ac}}{K_S+S_{ac}}X_{ac}I_3$
12	Uptake of Hydrogen								-1	$(1-Y_{h2})$	$-\sum_{i=1-9,11-24} C_i\nu_{i,12}$	$-(Y_{h2})\,N_{bac}$		$k_{m,h2}\frac{S_{h2}}{K_S+S_{h2}}X_{h2}I_1$
13	Decay of X_{su}													$k_{dec,Xsu}X_{su}$
14	Decay of X_{aa}													$k_{dec,Xaa}X_{aa}$
15	Decay of X_{fa}													$k_{dec,Xfa}X_{fa}$
16	Decay of X_{C4}													$k_{dec,Xc4}X_{c4}$
17	Decay of X_{pro}													$k_{dec,Xpro}X_{pro}$
18	Decay of X_{ac}													$k_{dec,Xac}X_{ac}$
19	Decay of X_{h2}													$k_{dec,Xh2}X_{h2}$
		Monosaccharides (kgCOD m^{-3})	Amino Acids (kgCOD m^{-3})	Long chain fatty acids (kgCOD m^{-3})	Total valerate (kgCOD m^{-3})	Total butyrate (kgCOD m^{-3})	Total propionate (kgCOD m^{-3})	Total acetate (kgCOD m^{-3})	Hydrogen gas (kgCOD m^{-3})	Methane gas (kgCOD m^{-3})	Inorganic Carbon (kmoleC m^{-3})	Inorganic nitrogen (kmoleN m^{-3})	Soluble inerts (kgCOD m^{-3})	Inhibition factors $I_1 = I_{pH}I_{IN,lim}$ $I_2 = I_{pH}I_{IN,lim}I_{h2}$ $I_3 = I_{pH}I_{IN,lim}I_{NH3,Xac}$

Table 4.3. Biochemical rate coefficients and kinetic rate equations for particulate components (adapted from Batstone *et al.* 2002).

Component → i	13	14	15	16	17	18	19	20	21	22	23	24	Rate (ρ_j, kg COD.m^{-3}.d^{-1})
j Process ↓	X_c	X_{ch}	X_{pr}	X_{li}	X_{su}	X_{aa}	X_{fa}	X_{c4}	X_{pro}	X_{ac}	X_{h2}	X_I	
1 Disintegration	-1	$f_{ch,xc}$	$f_{pr,xc}$	$f_{li,xc}$								$f_{xI,xc}$	$k_{dis}X_c$
2 Hydrolysis Carbohydrates		-1											$k_{hyd,ch}X_{ch}$
3 Hydrolysis of Proteins			-1										$k_{hyd,pr}X_{pr}$
4 Hydrolysis of Lipids				-1									$k_{hyd,li}X_{li}$
5 Uptake of Sugars					Y_{su}								$k_{m,su}\frac{S_{su}}{K_S+S}X_{su}I_1$
6 Uptake of Amino Acids						Y_{aa}							$k_{m,aa}\frac{S_{aa}}{K_S+S_{aa}}X_{aa}I_1$
7 Uptake of LCFA							Y_{fa}						$k_{m,fa}\frac{S_{fa}}{K_S+S_{fa}}X_{fa}I_2$
8 Uptake of Valerate								Y_{c4}					$k_{m,c4}\frac{S_{va}}{K_S+S_{va}}X_{c4}\frac{1}{1+S_{bu}/S_{va}}I_2$
9 Uptake of Butyrate								Y_{c4}					$k_{m,c4}\frac{S_{bu}}{K_S+S_{bu}}X_{c4}\frac{1}{1+S_{va}/S_{bu}}I_2$
10 Uptake of Propionate									Y_{pro}				$k_{m,pr}\frac{S_{pro}}{K_S+S_{pro}}X_{pro}I_2$
11 Uptake of Acetate										Y_{ac}			$k_{m,ac}\frac{S_{ac}}{K_S+S_{ac}}X_{ac}I_3$
12 Uptake of Hydrogen											Y_{h2}		$k_{m,h2}\frac{S_{h2}}{K_S+S_{h2}}X_{h2}I_1$
13 Decay of X_{su}	1				-1								$k_{dec,Xsu}X_{su}$
14 Decay of X_{aa}	1					-1							$k_{dec,Xaa}X_{aa}$
15 Decay of X_{fa}	1						-1						$k_{dec,Xfa}X_{fa}$
16 Decay of X_{c4}	1							-1					$k_{dec,Xc4}X_{c4}$
17 Decay of X_{pro}	1								-1				$k_{dec,Xpro}X_{pro}$
18 Decay of X_{ac}	1									-1			$k_{dec,Xac}X_{ac}$
19 Decay of X_{h2}	1										-1		$k_{dec,Xh2}X_{h2}$
	Composites (kgCOD m^{-3})	Carbohydrates (kgCOD m^{-3})	Proteins (kgCOD m^{-3})	Lipids (kgCOD m^{-3})	Sugar degraders (kgCOD m^{-3})	Amino acid degraders (kgCOD m^{-3})	LCFA degraders (kgCOD m^{-3})	Valerate and butyrate degraders (kgCOD m^{-3})	Propionate degraders (kgCOD m^{-3})	Acetate degraders (kgCOD m^{-3})	Hydrogen degraders (kgCOD m^{-3})	Particulate inerts (kgCOD m^{-3})	Inhibition factors $I_1 = I_{pH}I_{IN,lim}$ $I_2 = I_{pH}I_{IN,lim}I_{h_2}$ $I_3 = I_{pH}I_{IN,lim}I_{NH3,Xac}$

Where the *Input* and *Output* terms describe the mass flow across the system boundaries and are depending of the physical characteristics of the system modelled. The *Reaction* term groups specific processes, and the matrix represents the reactions for each component (*i*) and process (*j*) with the description of processes given in the left hand column and the component nomenclature on the last row. In the last column, the process rate (ρ_j) is given for each process and the remainder of each row is completed with the stoichiometric coefficients ($\nu_{i,j}$). The overall specific reaction term r_i for each component can be calculated by adding the products of the stoichiometric coefficients and process rates as defined in Equation 4.4 (Batstone *et al.* 2002):

$$r_i = \sum_j \upsilon_{i,j} \rho_j \qquad \textbf{(4.4)}$$

Where ρ_j, $\nu_{i,j}$ and r_i represent the process rate, stoichiometric coefficients and the overall specific reaction term respectively. An example of the application of Equation 4.4 is given below with the expression of the overall rate of reaction for long chain fatty acids (Equation 4.5):

$$r_3 = \underbrace{f_{fa,li}\left(k_{hyd,li} X_{li}\right)}_{\text{Hydrolysis of lipids}} - \underbrace{K_{m,fa} \frac{S_{fa}}{K_s + S_{fa}} X_{fa} I_2}_{\text{Uptake of long chain fatty acids}} \qquad \textbf{(4.5)}$$

Where $f_{fa,li}$ is the yield of fatty acids from lipids, $k_{hyd,li}$ is the first order parameter for the hydrolysis of lipids, X_{li} is the concentration of lipids degraders, $K_{m,fa}$ is the Monod maximum specific uptake rate for the uptake of fatty acids, S_{fa} is the total concentration of long chain fatty acids, K_s is the half saturation value for the uptake of

fatty acids, X_{fa} is the concentration of long chain fatty acids degraders, I_2 is the inhibition function for the uptake of fatty acids as expressed in Table 4.2. Liquid-gas transfer rates and acid-base reactions can also be implemented in a similar matrix form.

The mechanisms of inhibition considered in the model are reversible inhibition (non-competitive, uncompetitive and competitive forms), direct impact of the inhibitor on the microbial yield and decay, empirical inhibition forms for pH, competitive uptake and secondary substrate Monod kinetics (Batstone *et al.* 2002). With the exception of pH inhibition defined by specific equations, non-competitive inhibition is widely used in the model structure for free ammonia and hydrogen inhibition for example. The inhibition of all uptake processes (j=5-12 in Table 4.2 and Table 4.3) in the case of inorganic nitrogen shortage and pH inhibition are considered in the original model structure. Similarly free ammonia and hydrogen inhibition are implemented for the uptake of long chain fatty acids, volatile fatty acids and hydrogen (j=7-12). The uptake processes of valerate and butyrate are defined by a competitive uptake function (j=8-9). Inhibition forms are implemented as coefficients to Monod-type uptakes and can easily be modified to consider specific inhibition phenomenon such as a non-linear effect of the inhibitor concentration on the process kinetics or more particular inhibitory compounds (Fountoulakis *et al.* 2008; Fezzani and Ben Cheikh 2009).

4.2.4. Modelling of physico-chemical processes

Non-biologically mediated processes (see section 2.1.2) considered in the ADM1 are ion association/dissociation, acid-base reactions and liquid-gas transfer. Logarithmic values of acid dissociation coefficients (pKa) are considered in the model for

inorganic carbon, inorganic nitrogen, volatile fatty acids and hydrogen sulphide acid dissociation. Acid-base reactions are calculated using the charge balance approach and used for pH calculation (Batstone *et al.* 2002). The charge balance can be expressed such as Equation 4.6:

$$\sum S_{C^+} - \sum S_{A^-} = 0 \qquad \textbf{(4.6)}$$

Where sum of S_C^+ represents the total cationic equivalent concentration and sum S_A^- the total anionic equivalent concentration. The charge balance as implemented in the original model is expressed in Equation 4.7:

$$S_{Cat^+} + S_{NH_4^+} + S_{H^+} - S_{HCO_3^-} - \frac{S_{Ac^-}}{64} - \frac{S_{Pr^-}}{112} - \frac{S_{Bu^-}}{160} - \frac{S_{Va^-}}{208} - S_{OH^-} - S_{An^-} = 0 \qquad \textbf{(4.7)}$$

Where S_{NH4}^+ is the ammonium ion concentration, S_H^+ is the hydrogen ion concentration, S_{HCO3}^- is the bicarbonate ion concentration, S_{Ac}^-, S_{Pr}^-, S_{Bu}^- and S_{Va}^- are acetate, propionate, butyrate and valerate ions concentrations respectively with denominators representing the chemical oxygen demand content per charge, S_{OH}^- is the hydroxide ion concentration, S_{Cat}^+ and S_{An}^- represent the concentrations of metallic ions and are included to represent strong bases and acids respectively. S_{Cat}^+ and S_{An}^- are considered as inert compounds with no consumption or reaction terms.

Liquid-gas transfers are implemented for hydrogen, methane and carbon dioxide. Henry's law can be used to describe the equilibrium relationship between gas and liquid phases in contact. The ADM1 further considers resistance to transfer of

relatively insoluble gases and therefore introduces overall mass transfer coefficients (K_La) during liquid-gas transfer processes. The kinetic transfer rates of gas are implemented such as Equation 4.8:

$$\rho_{T,i} = k_L a(S_{liq,i} - K_H \rho_{gas,i}) \quad \textbf{(4.8)}$$

Where $\rho_{T,i}$ represents the kinetic transfer rate of the gas considered (carbon dioxide, hydrogen or methane), k_La is the dynamic liquid-gas transfer coefficient depending on mixing, temperature and liquid properties, K_H is the Henry's law coefficient, $S_{liq,i}$ and $\rho_{gas,i}$ are the liquid gas concentration and gas phase partial pressure respectively. The gas phase rate equations are otherwise implemented similarly to the liquid phase equations and the influence of temperature is also considered for physico-chemical processes since it has a fundamental influence on equilibrium coefficients.

Chapter 5
Summary of literature review

This section summarises the themes identified in the overview of the literature considered in the review chapters. It includes the main limitations identified from the literature for the uptake of anaerobic digestion of seaweed and its mathematical modelling. It provides justifications for the work carried out and the experimental and modelling approaches used. !

The fundamentals of anaerobic digestion have been theoretically formulated and provided with considerable empirical evidence. The process mechanisms are now understood to such a level that they can be reproduced in a controlled fashion. Moreover, the understanding of successive and often simultaneous biochemical and physico-chemical processes has led to the development of innovative ways to stimulate and improve the anaerobic digestion of numerous sources of organic material. Developments in microbiology, environmental engineering and industrial process control have further promoted the uptake of anaerobic digestion as both a sustainable way to produce clean energy and a solution for mitigating the ever increasing burden of anthropogenic wastes on the environment. Many studies have demonstrated the potential of anaerobic digestion, and the fact that the process is gradually gaining acceptance is inferred. However, anaerobic digestion seems to still lack the reliability and effectiveness of other energy yielding processes and its use as

an energy source *per se* might be jeopardised in an economically competitive environment. The paucity of suitable organic matter, which is indispensable to guarantee the process stability, epitomises one of the issues associated with anaerobic digestion. Interest has thus grown on the anaerobic digestion of alternative sources of organic material in combination with more standard substrates.

In principle, the anaerobic digestion of seaweed represents an acceptable solution to fulfil the requirements of degradable organic matter. To this extent, brown seaweed seems particularly of interest in the UK because of its prevalence on Scottish coasts and its potentially good biodegradability. Omitting technical, environmental and perhaps ethical issues resulting from the extensive harvesting of brown seaweed, relatively high organic matter content associated with small quantities of refractory compounds are seen as favourable factors when considering its potential for biodegradation. Despite all the aforementioned aspects, brown seaweed species are characterised by inhibitory substances that could be prejudicial to their successful anaerobic digestion. At the core of the problem lies high concentrations of salt associated with the effects of a unique type of phenolic compound referred to as phlorotannins. Strategies to limit the effect of salinity on microorganisms are well documented, but there is little evidence on their performance in systems with varying levels of salt as often encountered with a typical substrate rotation scheme. The impact of phlorotannins on anaerobic microorganisms also requires a more detailed study and would extend the work already conducted on their effect on other common bacteria such as *Escherichia coli*.

The mathematical modelling of anaerobic digestion has seen important developments in the past decades. From models considering only one biochemical process to models in which multiple biochemical and physico-chemical processes

associated with a variety of microorganisms are considered, the discipline can now be considered as mature. The Anaerobic Digestion Model No.1 has been a key milestone for the wider application and acceptance of modelling tools applied to anaerobic digestion systems. It has been used as a platform for innumerable applications and has been found particularly reliable for process simulation and design. The model open framework, which allows the addition of processes not originally included in its structure, even further broadens the relevance of the model. However, the introduction of a complex model has given rise to a new set of problems relating to the estimation and determination of its extensive number of parameters. A multitude of methods have been published with regard to the determination of the model inputs but no techniques have yet been set to determine which parameters most need to be accurately determined. This observation finds even more significance in a context in which the model is implemented as a system of differential equations where initial conditions are critically important for model behaviour and predictions, but are often difficult to determine experimentally. Systematic mathematical methods, such as parametric sensitivity analysis would thus be potentially suitable with respect to resolving this issue.

The experimental work discussed in the subsequent chapters was designed to assess the effect of salinity and biomass salinity adaptation on anaerobic processes. The impact of phenolic compounds on anaerobic microorganisms was also investigated. Co-digestion of brown seaweed was investigated in a laboratory-scale anaerobic digester using *Laminaria digitata*. A modelling approach was further used in an attempt to reproduce the mechanisms observed experimentally, and obtain reliable predictions of the system behaviour. Due to the intrinsic complexity of the modelling platform used throughout this work, parametric sensitivity analysis was a pre-requisite for model extension and calibration.

Chapter 6
General materials and methods

This section introduces the general materials and methods used for the experimental and modelling aspects of the study. This includes a detailed description of reactor and batch systems, experimental designs, analytical methods and modelling tools. Details on the accuracy, repeatability and precision of analytical methods are also provided.

6.1. Experimental designs

6.1.1. Continuous reactor system

Laboratory-scale anaerobic digestion experiments were conducted in a mixed reactor as shown in Figure 6.1. The reactor had an 8 litre total capacity with 5 litre effective capacity. Temperature was automatically controlled, and heating was achieved through an insulated electrical heating wire wrapped around the outside of the vessel with the temperature being monitored in real time using an electronic thermometer (Invensys controls, Italy). Intermittent mixing (15 seconds every 20 minutes) was achieved through the use of a propeller attached to a stepper motor (Igarashi IG33, Trident Engineering, UK) controlled automatically. Feeding was done manually or automatically using a peristaltic pump (Masterflex L/S: Cole-Parmer, UK) through a port located at the top of the main vessel and connected to the liquid phase of the reactor to prevent gas leakage. Similarly, the effluent was withdrawn manually through a valve located at the base of the digester or automatically using a peristaltic

pump connected to the bottom of the reactor through the feeding port. Peristaltic pumps used for daily feeding were controlled by electronic timers. Gas composition was monitored from the headspace through a gas-tight sampling port. The vessel was connected to a gas collector made of two cylinders and based on the water displacement principle.

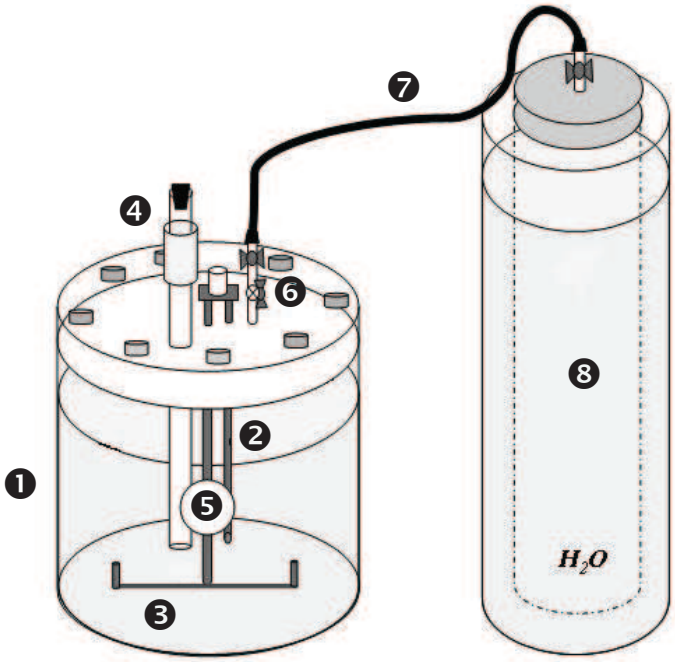

Figure 6.1. Schematic representation of the laboratory scale reactor. Temperature controlled vessel (❶), thermometer probe for temperature control (❷), propeller attached to a stepper motor (❸), feeding port (❹), outlet valve (❺), sampling port (❻), flexible tubing connected to the gas outlet (❼) and gas collector (❽).

6.1.2. Batch reactor system

Anaerobic batch tests were conducted using either 2 litre or 500 ml serum bottles. Specific inoculum and substrate volumes (ratio 4:1 respectively) were used in each assay, but the final liquid volume was kept similar in each bottle (500 ml or 125 ml) in order to ensure a sufficient headspace volume and therefore prevent the build up of pressure. The bottles were placed in an incubator to maintain mesophilic temperatures

(37°C±1°C) and closed with rubber caps (Fisher, UK). The headspace of each bottle was then flushed with N_2 for about 2 minutes to guarantee anaerobic conditions. Gas sampling was conducted by inserting a 100 µL gas tight syringe through the rubber cap as shown in Figure 6.2. In order to avoid overpressure in the bottles, gas release was carried out routinely by displacement of a syringe piston and the amount of methane gas released was added to cumulative biogas production. Bottles were mixed manually for about a minute on a daily basis.

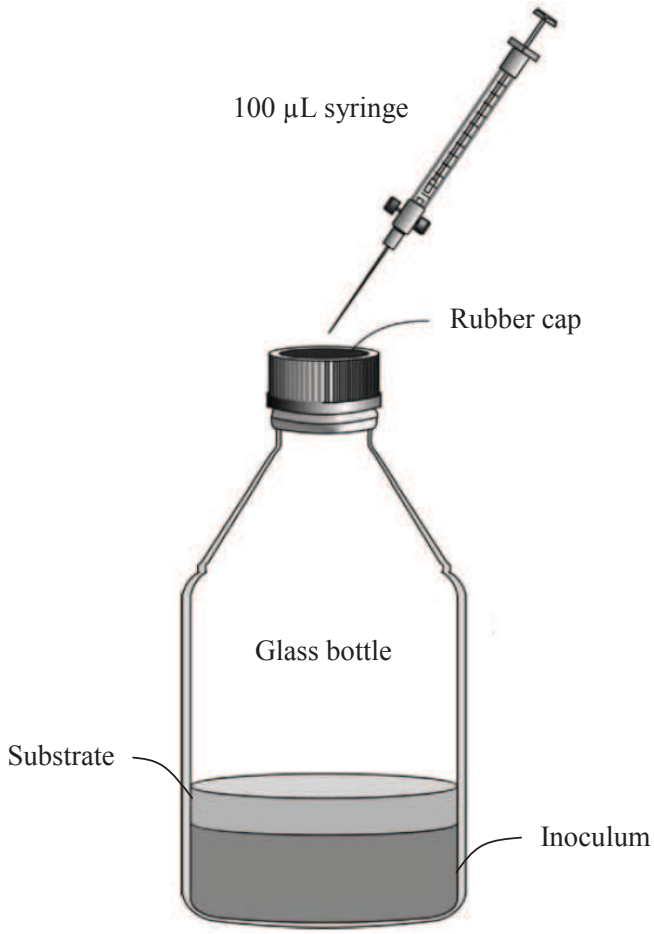

Figure 6.2. Illustration of batch assays and gas sampling (adapted from Hansen *et al.* 2004).

6.1.3. Cell membrane leakage assay

Cell membrane leakage was assessed by measuring potassium fluxes and 260 nm absorbing material concentrations over time in a solution consisting of mixed

anaerobic microorganisms and phenolic compounds at different concentrations. In order to determine potassium ion (K^+) leakage, 8 ml of anaerobic digested sludge was placed in a beaker and magnetically stirred at room temperature. At the beginning of the assay, a volume of 2 ml of phenol solution was added to give a final known reaction concentration in 10 ml. The potassium concentration was determined by flame emission spectroscopy with a Flame Photometer 400 (Ciba-Corning, USA) calibrated with potassium chloride (KCl) standards at different ionic strengths. Potassium levels were measured at time intervals of three minutes by transferring 2 ml of sample in Eppendorf tubes. The tubes were centrifuged at 2000×*g* for 2 minutes and 1 ml of supernatant was transferred in a clean vial for measurement. Results were converted to concentrations of K^+ ions by referring to a conversion graph constructed using three KCl standard solutions (0.001-0.1 M).

In order to determine the leakage of 260 nm absorbing material, the same procedure was repeated with a final reaction volume of 5 ml. Samples were taken every five minutes and 1 ml of supernatant was diluted with the same volume of deionised water and transferred into a quartz UV cuvette. The absorbance of the cuvette was read at 260 nm in a calibrated spectrophotometer (DR5000: Hach-Lange, USA).

6.1.4. Cell suspension preparation for microscopy analysis

Mixed microbial cultures were grown in a nutrient media under anaerobic conditions for 24 hours at mesophilic temperatures (37°C±1°C) and 125 ml was transferred to vials and mixed with 25 ml of a sodium acetate solution (25 g/l). The cultures were then incubated under anaerobic conditions for a further 7 days before being exposed to phenolic compounds or water (control) for 5 hours and centrifuged at 3000×*g* for

20 minutes. Pellets collected were washed twice with a solution of 0.9% w/v sodium chloride (NaCl) in sterile distilled water to remove the presence of the test materials. The final pellets were re-suspended in glutaraldehyde (2.5% v/v solution in normal saline) and incubated at room temperature for fixation. The fixed pellets were then suspended in osmium tetraoxide solution (0.2% OsO_4 in water) and left to fix/stain overnight. The suspensions were centrifuged (4000×*g* for 15 min) and rinsed twice with distilled water. Uranyl acetate solution (2% aq.) was then added to the pellets and left overnight. The pellets were washed twice with distilled water. Cells were dehydrated through a graded ethanol series with two changes in absolute ethanol. The pellets were then placed in propylene oxide (100%) and infiltrated with a 1:1 (v/v) mixture of propylene oxide and Durcapan resin (Sigma, UK) on a rotary wheel (4 rev. min^{-1}) for 24 h at room temperature. The pellets were then infiltrated with 100% Durcapan resin as above overnight. Pellets were transferred to flat embedding moulds with fresh resin and placed in a 60°C oven for 24 h to polymerise the resin. Sections were cut from the resin-fixed pellets using a Reichert OMU-3 ultramicrotome fitted with a diamond knife and mounted/collected on 50 mesh pioloform coated copper grids. After staining with uranyl acetate and lead citrate, sections were examined using a JEOL-1200 EX transmission electron microscope (Baloch *et al.* 2008).

6.2. Analytical methods

The methods introduced here are characterised through their precision, repeatability and accuracy expressed in percentages. The standard deviation of a set of measurements is commonly used to obtain the precision of analytical methods. Precision is defined as the closeness of agreement between independent test results obtained under stipulated conditions (ISO 5725 1994). When a confident reference is

available, the method accuracy can be calculated as the closeness of agreement between a test result and the accepted reference value (ISO 5725 1994). The repeatability of an analytical method is defined as the uncertainty of repeated measurements of the same sample within the same analytical series (ISO 5725 1994) and can be expressed as Equation 6.1:

$$r = 1.96 \cdot \sqrt{2} \cdot s_r \qquad \textbf{(6.1)}$$

Where S_r is the standard deviation or precision within samples. When reliable standards could not be used or when the number of replicates was not large enough for statistical calculations, precision and repeatability were obtained from manufacturer guidelines.

6.2.1. Biogas production and composition

Biogas production from the reactor system was measured daily and was based on the water displacement theory following Archimedes' Principle. The biogas production was measured using a height gasometer made of two cylinders of different diameters as shown in Figure 6.1 (❽). One cylinder was closed and partially submerged in an open container of the barrier solution (Walker *et al.* 2009). Gas was introduced into the column from the reactor and displaced the barrier solution (i.e. water) into this collector. The volume of gas introduced was simply calculated by measuring the change in liquid height in the column (Walker *et al.* 2009) using Equation 6.2:

$$v = \frac{\pi \cdot r^2 \cdot h}{1000} \qquad \textbf{(6.2)}$$

Where *r* and *h* are the cylinder measured radius and height respectively, in cm and *v* the volume of gas in litres. The calculated precision of the biogas production measurement is 1.9% with a repeatability of 5.1%. Accuracy was not calculated. Methane (CH_4,), carbon dioxide (CO_2), and oxygen (O_2) levels were measured using a gas analyser (GA5000: Geotechnical Instruments, UK) which also records indicative concentrations of carbon monoxide (CO) and hydrogen sulphide (H_2S). Methane and carbon dioxide were evaluated by dual wavelength infrared cells with reference channels at an absorption wavelength of 3.4 microns and 4.26 microns respectively. Oxygen, carbon monoxide and hydrogen sulphide levels were determined by internal electrochemical cells composed of noble metal electrodes in an electrolyte usually made of strong inorganic acids. The typical accuracy given by the manufacturer is 3% for CH_4 and CO_2, 1% for O_2 and 10% for CO and H_2S. The analyser was calibrated every 6 months by the manufacturer. Methane yields were also determined by gas chromatography with a Hewlett-Packard 5890 Series II gas chromatograph with dual thermal conductivity detector and an AT-Alumina stainless steel capillary column. Injector, oven and detector temperatures were 120°C, 50°C and 150°C respectively and helium was used as a carrier gas at a flow of 7 ml. min^{-1}. Standard of 50%, 30%, 20%, 10% and 1% CH_4 (balanced with nitrogen) were used for calibration. The accuracy of the analytical equipment was found to be equal to 12% with a precision of 1% CH_4. The repeatability was calculated as equal to 3%.

6.2.2. pH value

The pH values were measured using a *Sension 3* laboratory pH meter (Hach, USA). The method is based on the voltage changes induced by different concentrations of hydrogen cations (H^+) measured by an electrode usually made of silver in an

electrolyte solution. Since the pH is determined by taking the negative logarithmic value of the hydrogen ions in a solution, the pH meter determines a slope from the measurements of standard pH solutions (standard millivoltage) and subsequent measurements are hence converted directly to pH values. In order to ensure accuracy, the pH meter is calibrated using three standard solutions (pHs 4, 7 and 10). The precision, repeatability and accuracy of the measurements are highly dependent of the pH probe. The manufacturer guaranties an accuracy of ±0.2% of the reading for the equipment used in this work.

6.2.3. Volatile fatty acids

The method used for the measurement of volatile fatty acids (VFA) is based on the esterification of the carboxylic acids existing in the sample and determination of the esters by the ferric hydroxamate reaction. The concentration of total VFA converted to acetic acid (AcOH) was then estimated spectrophotometrically at a wavelength of 495 nm. A more detailed procedure can be found from Montgomery *et al.* (1962). The precision and repeatability of the method were calculated on the basis of seven series of samples measured in triplicates in the best possible conditions and found to be equal to 4.1% and 11.4% respectively.

6.2.4. Partial and total alkalinity

The measurement of alkalinity is based on the principle that hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Thus, the alkalinity of a sample is its acid-neutralising capacity as the sum of all the titratable bases reported as calcium carbonate per litres ($CaCO_3$/l) (APHA 1992). The most current method to determine the alkalinity of a sample is to

titrate a solution of this sample and methyl orange pH indicator with a solution of hydrogen chloride (HCl). However, the precipitation of the solution indicating that a sufficient quantity of acid has been added is subjective and often biased. Moreover, it is not possible to determine partial alkalinity by using a colour indicator. Hence, alkalinity was determined by the titration of a sample with acid until pH reaches 5.75 and 4.3 for partial and total alkalinity respectively. Alkalinity expressed as mg $CaCO_3$/l is then determined using Equation 6.3:

$$Alkalinity = \frac{V_{acid} * N * 50000}{V_{sample}} \qquad \textbf{(6.3)}$$

Where V_{acid} is the volume of standard acid used in ml, V_{sample} the volume of sample in ml and N the normality of standard acid. Based on a set of measurements of the same sample within the same analytical series, the precision of alkalinity measurements was found equal to 1% with a repeatability of 2.6%. The precision of the measurements is however strongly dependent on the precision of the pH meter and probe used. Accuracy was not calculated.

6.2.5. Total and volatile solids

The quantification of total and volatile solids from a sample is conducted according to Standard Methods (APHA 1992), method 2540. Total solids (TS) are measured from a representative liquid sample in a weighted dish and dried to constant weight for up to 24 hours in an oven at 105˚C. The volume of sample taken for measurement was carefully measured and total solids are calculated in g/l using Equation 6.4:

$$TS = \frac{(A-B)*1000}{V_{sample}} \qquad \textbf{(6.4)}$$

Where A is the weight of the dried residue and dish in grams, B the weight of the dish alone in grams, and V_{sample} the volume of sample in ml. The residual sample after total solids determination was ignited at 550˚C in a furnace for up to 2.5 hours until a constant weight was reached. The concentration of volatile solids (VS) can then be calculated in g/l by using Equation 6.5:

$$VS = \frac{(A-C)*1000}{V_{sample}} \qquad \textbf{(6.5)}$$

Where C is the weight of the sample and dish in grams after ignition. Volatile solids content can also be expressed as a percentage of TS. Precision and repeatability for total solids are 6.7% and 20% respectively. For volatile solids, precision and repeatability are found equal to 10.7% and 30% respectively. Measurements were conducted in triplicate. The accuracy of the method was mainly dependent on the pipetting method used.

6.2.6. Ammonium nitrogen

The quantification of ammonium nitrogen (NH_4) used in this work relies on the principle that ammonium ions (NH_4^+) react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside and forms indophenol blue in the presence of ammonium nitrogen. The colorimetric change can then be evaluated by reading the absorbance at 694 nm with a spectrophotometer and results are

converted in NH_4-N. The concentration of ammonium nitrogen was determined using cuvette tests LCK 304 (Hach-Lange, USA). The accuracy of the method conducted on an accepted standard in triplicate was found to be 8% with a precision of 3%. An estimation of the unionised form of nitrogen can be calculated from the formula given by McCarty and McKinney (1961) and expressed in Equation 6.6:

$$[NH_3] = 1.13 \cdot 10^{-9} \cdot \frac{[NH_4]}{[H^+]} \qquad \textbf{(6.6)}$$

The concentration of hydrogen ions can be calculated as $[H^+] = 10^{-pH}$.

6.2.7. Sulphate and sulphide

The measurement of sulphate relies on the principle that sulphate ions react with barium chloride ($BaCl_2$) to form barium sulphate ($BaSO_4$) in an aqueous solution. However, $BaSO_4$ is only sparingly soluble and the turbidity resulting from the presence of sulphate ions (SO_4^{2-}) is measured photometrically at 435 nm. The accuracy of the method conducted on an accepted standard was found equal to 4.3% with a precision of 1.8% using cuvette tests LCK 353 (Hach-Lange, USA).

Total sulphides in the form of hydrogen sulphides (H_2S and HS^-) and certain metal sulphides react with N,N-dimethyl-p-phenylenediamine sulphate to form methylene blue in the presence of sulphide. The colour change is measured photometrically at 665 nm. The accuracy of the method conducted on an accepted standard was found equal to 16% with a precision of 5%.

The concentration of H_2S can be determined by multiplying the total dissolved sulphide concentration by a correction factor knowing the pH of the solution (LaMotte, USA).

6.2.8. Phosphorous

The measurement of total phosphorous is based on the principle that phosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex reduced by ascorbic acid to phosphomolybdenum blue. The colour change was evaluated by reading absorbance at 850 nm with a spectrophotometer and results were converted to PO_4-P for orthophosphate. If the sample is hydrolysed for an hour at 100˚C before the addition of ascorbic acid, the total phosphorous content can be measured. The accuracy of the method conducted on an accepted standard was found to be 4.4% with a precision of 1.8% using cuvette tests LCK 049 (Hach-Lange, USA).

6.2.9. Total organic and inorganic carbon

The measurement is based on the principle that total carbon and total inorganic carbon contents from a liquid sample can be converted to carbon dioxide (CO_2) by oxidation and acidification respectively. Carbon dioxide was transferred from the reaction liquid to an indicator solution and colour change was measured at 435 nm to determine the results after the samples have been heated at 100˚C for 2 hours. Total organic carbon was determined by subtracting the inorganic carbon concentration from the amount of total carbon measured from the same sample. The accuracy of the method, as determined by the manufacturer, was found equal to 5.7% with a precision of 2.5% using cuvette tests LCK 381 (Hach-Lange, USA).

6.2.10. Chemical oxygen demand

To determine the chemical oxygen demand (COD) concentration, the sample was heated at 150°C for two hours with a strong oxidising agent (potassium dichromate). Organic compounds that can be oxidised react by reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}). A spectrophotometer was then used to evaluate the sample colour change by reading absorbance at 620 nm hence determining the amount of Cr^{3+} produced with results converted to mg $COD.l^{-1}$ or mg O_2/l. The accuracy of the method conducted on an accepted standard was found equal to 6.5% with a precision of 2.7% using cuvette tests LCK 014 (Hach-Lange, USA).

6.2.11. Cations

The cations of main sea salts, i.e. sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+), were measured in an aqueous solution by using flame atomic emission spectrometry, commonly referred to as flame photometry. The energy of the flame used is sufficient to produce free atoms and then excite them to higher energy levels, thus making them visible and quantifiable at different emission lines (Machve 2007). Na^+, Ca^{2+}, and K^+ emission lines are clearly separated at 589 nm, 649 nm and 766 nm respectively and can be evaluated through flame photometry. The flame photometer used in this work (Model 400: Ciba-Corning, USA) was fitted with tinted glass filters and results were converted on a scale by photo-detectors after calibration with three appropriate standards and creation of a calibration curve. The standards used were made of distilled water and known concentrations of the different cations in solution at 0.1M, 0.01M and 0.001M. The precision of the measurement for Na^+, Ca^{2+} and K^+ based on a set of samples from the same analytical series is 5.3% standard deviation and 15.3% repeatability for all three cations with the precision of the method depending mainly

on the precision of the standard solution used for calibration. Magnesium ions (Mg^{2+}) were measured using an atomic absorber (AAnalyst 200: Perkin-Elmer, USA) since the number of atoms excited to emission in a flame is very small for magnesium. The absorption device is similar to a flame photometer but a source of radiation traverse the flame since all atoms are capable of absorbing radiation of their resonance wavelength (Machve 2007). The precision of the measurement for Mg^{2+} is 3.2% with a repeatability of 8.9%. The associated accuracy with an accepted standard is 2.4% using a magnesium Atomax Hollow cathode lamp (Perkin-Elmer, USA).

6.2.12. Phenolic compounds

Polyphenolic compounds extracted from brown seaweed, usually referred to as phlorotannins, were determined using the DMBA (2,4-dimethoxy benzaldehyde) assay (Stern *et al*. 1996a). The measurements were conducted after the samples were incubated for an hour at 30˚C with N,N-dymethylformamide, 16% HCl and a solution of DMBA and glacial acetic acid. Standard solutions were made with anhydrous phloroglucinol (Sigma, UK) dissolved in deionised water. The method is based on the principle that in the presence of 1,3- and 1,3,5-substituted phenols such as phlorotannins, 2,4-dimethoxy benzaldehyde reacts specifically by creating a colour change. The colour change was evaluated by reading absorbance at 510 nm with a spectrophotometer and results were converted to phlorotannins concentrations by converting the absorbance results using a standard curve constructed with five phloroglucinol standard solutions (maximum absorbance 2.5 units). The advantage of the DMBA method is the absence of interference from non-phlorotannin substances (Stern *et al*. 1996a). However, there is a strong variation in reactivity of different fractions of phlorotannins presumably due to their different chemical structure, hence

being a disadvantage of the assay (Koivikko 2008). This inaccuracy can be avoided by using a specific standard for each species analysed (Stern *et al*. 1996a). Yet, none of the 22 DMBA assays reviewed by Koivikko (2008) reported the use of standards derived from the seaweed itself and phloroglucinol is commonly used. The results can be converted into % of phlorotannins per dry weight of the sample. The method precision was found equal to 14% with a repeatability of 40%. The associated accuracy with an accepted standard was 0.8%.

6.3. Operational parameters calculations

6.3.1. Organic loading rate

The organic loading rate (OLR) represents the quantity of substrate introduced into the anaerobic system in a given period of time with the substrate being defined either by its total solids, volatile solids or chemical oxygen demand concentrations. The OLR, expressed in kg TS/m^3 reactor per day, can be calculated by using Equation 6.7:

$$OLR = \frac{Q \times S}{V_{reactor}} \qquad \textbf{(6.7)}$$

Where Q is the substrate flow rate in m^3/day, S the substrate concentration in the inflow expressed in kg TS/m^3 and $V_{reactor}$ the effective volume of the anaerobic reactor in m^3.

6.3.2. Hydraulic and solids retention times

The hydraulic retention time (HRT) represents the time that a fluid element theoretically spends in an anaerobic reactor and is calculated in days knowing the reactor volume and flow rate of the effluent substrate as expressed in Equation 6.8:

$$HRT = \frac{V_{reactor}}{Q} \qquad \textbf{(6.8)}$$

Where $V_{reactor}$ is the effective volume of the reactor in m^3 and Q the substrate flow rate in m^3/day. The residence time of solids (SRT) is expressed as the ratio between the content of solids in the reactor and the effluent solids flow rate and can be calculated in days using Equation 6.9:

$$SRT = \frac{V_{reactor} \times X}{W} \qquad \textbf{(6.9)}$$

Where $V_{reactor}$ is the effective volume of the reactor in m^3, X the volatile solids concentration in the reactor in kg VS/m^3 and W the effluent solids flow rate expressed in kg VS/day. When the quantity of biomass extracted from a reactor is found to be equal to the biomass produced within the system, the solids concentration in the reactor will be constant in a given time and hence, the system is operating at steady-state conditions (Cecchi *et al.* 2003). However, for an ideal, completely stirred tank reactor in which the liquid phase is perfectly homogenous, the solid retention time is considered equal to the hydraulic retention time.

6.3.3. Gas production rate and specific gas production

The gas production rate (GPR) represents the biogas produced considering the volume of the reactor in a given period of time and is expressed in $m^3_{gas}.m^{-3}_{reactor}.day^{-1}$ as calculated by Equation 6.10:

$$GPR = \frac{Q_{biogas}}{V_{reactor}} \qquad \textbf{(6.10)}$$

Where Q_{biogas} is the biogas flow rate in m^3/day and $V_{reactor}$ the effective volume of the reactor in m^3. The specific gas production (SGP) is a measure of the biogas produced by unit of mass of substrate based on the volatile solids content of the inflow. This parameter can be used to compare the performances of different anaerobic systems since it considers the amount of biodegradable substrate introduced in the reactor. It is usually expressed in m^3 gas/kg VS fed and calculated by using Equation 6.11:

$$SGP = \frac{Q_{biogas}}{Q * S} \qquad \textbf{(6.11)}$$

Where Q_{biogas} is the biogas flow rate in m^3/day, Q the substrate flow rate in m^3/day and S the substrate concentration in the inflow expressed in kg VS/m^3.

6.3.4. Organic matter removal efficiency

Different equations can be found to measure the organic removal efficiency (η), which differs if the measurement of the substrate is made in terms of COD or VS. When the substrate conversion is measured as unit of volatile solids, the approach

used by Ross *et al.* (1992) can be employed to consider solely the biodegradable fraction of the substrate and the removal efficiency can be expressed in % as in Equation 6.12:

$$\eta = \frac{VS_{in} - VS_{out}}{VS_{in} - (VS_{in} \times VS_{out})} \times 100 \qquad \textbf{(6.12)}$$

Where VS_{in} is the percentage of volatile matter in the inflow and VS_{out} is the percentage of volatile matter in the effluent both being expressed in percentage.

6.3.5. Theoretical methane yield

The theoretical methane potential for a substrate with a known elemental composition can be estimated by the Buswell formula developed by Buswell and Boruff (1932). The formula was later modified by Boyle (1977) to include elemental nitrogen and sulphur and therefore obtain an estimation of the fraction of ammonia and hydrogen sulphide in the biogas as expressed in Equation (6.13):

$$C_aH_bO_cN_dS_e + \frac{1}{4}(4\cdot a - b - 2\cdot c + 3\cdot d + 2\cdot e)H_2O \rightarrow \frac{1}{8}(4\cdot a - b + 2\cdot c + 3\cdot d + 2\cdot e)CO_2 +$$

$$\frac{1}{8}(4\cdot a + b - 2\cdot c - 3\cdot d - 2\cdot e)CH_4 + dNH_3 + eH_2S \qquad \textbf{(6.13)}$$

The specific theoretical methane yield in litres methane (CH_4) per gram of volatile solids can then be estimated using Equation (6.14).

$$Y_{CH_4}^{Theoretical} = \frac{22.4 \cdot (4 \cdot a + b - 2 \cdot c - 3 \cdot d - 2 \cdot e)}{8 \cdot (12 \cdot a + b + 16 \cdot c + 14 \cdot d + 16 \cdot e)} \quad \textbf{(6.14)}$$

Furthermore, the methane to carbon dioxide ratio r_G can be calculated from the average carbon oxidation state (Harris and Adams 1979; Sialve *et al*. 2009; Lübken *et al.* 2010) as expressed in Equations 6.15 and 6.16.

$$r_G = \frac{CH_4}{CO_2} = \frac{4 - n}{4 + n} \quad \textbf{(6.15)}$$

With:

$$n = \frac{-b + 2 \cdot c + 3 \cdot d}{a} \quad \textbf{(6.16)}$$

The Buswell formula considers that all the matter contained in the substrate is biodegradable and converted to methane and does not allow the differentiation between the biodegradable and refractory fractions (Lesteur *et al.* 2010). In addition, the fraction of biodegradable matter used for biomass synthesis and potential inhibitory compounds are not taken into account. The theoretical methane yield is therefore always overestimating the experimental biomethanization potential. From the ultimate analysis results obtained in % by weight, the coefficients in the Buswell Equation (6.13) *a*, *b*, *c*, *d*, *e* can be calculated by dividing the proportion of weights by the atomic weights of the associated element.

6.3.6. Methane production rate constant

Considering that the cumulative methane production curve obtained during anaerobic batch tests follows a first-order rate, it is possible to estimate the methane production rate constant by means of Equation 6.17 as described by Gunaseelan (2004).

$$B(t) = B_0 \times (1 - e^{(-k_{dis} \cdot t)}) \qquad \textbf{(6.17)}$$

Where B_0 and K_{dis} represent the maximum methane yield and the disintegration rate constant in litres CH_4/gVS_{added} and day^{-1} respectively. The disintegration rate constant K_{dis} can then be estimated by taking the reciprocal of time from the start of the biodegradation assay until the biogas measurements equal 63.2% of the final biogas production (Gunaseelan 2004).

This value can also be determined more precisely by solving a nonlinear curve-fitting problem using the method of least-squares. The method consists in finding the coefficients x that solve the problem expressed by Equation 6.18:

$$\min_x \left\| F(x, t_{days}) - B_{prod} \right\|_2^2 = \min_x \sum_i (F(x, t_{days_i}) - B_{prod_i})^2 \qquad \textbf{(6.18)}$$

Given the input data t_{days} and the observed biogas production B_{prod} where $F(x, t_{days})$ is a function of the same size as B_{prod}.

6.4. Substrate preparation and source of inoculum

The substrates used during both reactor studies and batch assays have been prepared following the same procedure. Green peas (*Pisum sativum*) were obtained from commercially available sources. Brown seaweed *(Laminaria digitata*) was collected

from the Westhaven beach (56° 30′ N, 2° 42′ W) near Dundee, Scotland, UK in October 2010 and October 2011. After collection, the seaweed was washed with tapwater and several rinses were required to remove debris, sand and excess seawater. Both substrates were oven-dried at about 75°C for 24 hours and milled in an industrial blender (Fritsch, Germany) to reduce particle size to a maximum of 1 mm and obtain a homogenised feedstock. The substrates were then stored in sealed containers at room temperature.

The source of inoculum used for reactor start-up and batch assays was anaerobically digested sludge provided by the Hatton wastewater treatment plant (Hatton, Angus, UK) operating at mesophilic temperatures. A saline-tolerant source of inoculum was also used in one specific assay. The salt-tolerant inoculum originated from a laboratory scale anaerobic digester operating at mesophilic temperatures and treating seaweed with sodium levels of about 15 g Na^+/l for over 2 years prior to the collection of inoculum, and was therefore well adapted.

6.5. Extraction of phenolic compounds

Polyphenolic compounds, referred to as phlorotannins, were extracted from the brown seaweed *Laminaria digitata* after the substrate was prepared as explained above. The extraction of phenolic compounds from plant material requires extractants with different polarities and the general recommendation is to use a mixture of water and methanol, ethanol or acetone (Waterman and Mole 1994). Koivikko (2008) assessed the efficiency of different solvents for the extraction of phlorotannins and found that a solution of acetone and water was the most efficient. The algal powder obtained after substrate preparation was then extracted three times with a 7:3 acetone:water solution and let to evaporate in a fume hood. The resulting aqueous phase was frozen, freeze-

dried and dissolved into water to obtain a stock phlorotannin solution with a known concentration.

6.6. Modelling tools

The implementation of the ADM1 in Matlab/Simulink (MathWorks Inc., USA) carried out by Rosen and Jeppsson (2006) was used and subsequently modified to add new inhibition functions and co-digestion modules. Each unit of the model is represented by a system function (S-function) incorporated in Matlab through Simulink, which provides an interactive graphical interface. The files containing the model code were compiled and converted to Matlab executable (MEX) files before being used by Matlab. The model was implemented as a differential algebraic equation system to reduce stiffness resulting from the large range of time constraints. The implementation used also extended stoichiometry to guarantee mass balances for carbon and nitrogen and modified default values for some parameters. Calculations of pH and hydrogen levels were implemented as algebraic equations. SimLab was used for uncertainty and sensitivity analysis and developed by the Joint Research Centre of Ispra, Italy. Parameter optimisation was conducted using the Levenberg-Marquardt technique (Levenberg 1944; Marquardt 1963). The Levenberg-Marquardt method (LMA) is often used in minimisation problems occurring in least-squares regression analysis, i.e. the determination of an overall solution minimising the sum of the squares of the errors made in solving a single equation in a set of equations (Jaiswal and Khandelwal 2009). The method aims at optimising the parameter β of the model curve f(x, β) in order to minimise the sum of the squares of the deviations as expressed in Equation 6.19:

$$S(\beta) = \sum_{i=1}^{m} \left[y_i - f(x_{i,}\beta) \right]^2 \qquad \textbf{(6.19)}$$

Where y_i and x_i are pairs of independent and dependent variables respectively. LMA combines advantages of two other local optimisation techniques, namely the *steepest descent* and the Gauss-Newton based on the original method of Newton to find roots of complex equations. LMA, as an iterative method, starts similarly to a *steepest descent* technique and gradually becomes a Gauss-Newton method when getting closer to the optimum and is hence more robust and achieves better convergence than the two previous methods (Donoso-Bravo 2011). From the Levenberg-Marquardt method, it is possible to obtain the Jacobian matrix, which represents a matrix of all first-order partial derivates of a vector-valued function respective to another vector as expressed in Equation 6.20:

$$J = \begin{bmatrix} \frac{\partial y_1}{\partial x_1} & \cdots & \frac{\partial y_1}{\partial x_n} \\ \vdots & \ddots & \vdots \\ \frac{\partial y_m}{\partial x_1} & \cdots & \frac{\partial y_m}{\partial x_n} \end{bmatrix} \qquad \textbf{(6.20)}$$

When considering a function F: $R^n \rightarrow R^m$ is a function from the Euclidean n-space to m-space given by m real (as opposed to complex) component functions $y_1(x_1,...,x_n),...,y_m(x_1,...,x_n)$. If existing, all partial derivates of these functions can be organised in the Jacobian matrix as expressed above. This matrix can be further used to obtain an estimate of the accuracy of the parameters and confidence intervals for the newly determined parameters. These results can in turn provide an insight on the confidence of the model predictions. Once the optimisation has been achieved, it is

possible to compute the residuals between experimental values and model output with the calibrated set of parameters. The standard deviation of these residuals, also called the standard error of estimates (SEE), can be determined by Equation 6.21:

$$SEE = \sqrt{\frac{\sum residuals^2}{n_{data} - n_{parameters}}} \qquad \textbf{(6.21)}$$

Where n_{data} is the number of data points considered and $n_{parameters}$ the number of parameters taken into account in the optimisation process. From both previous calculations, the covariance matrix representing the covariance, i.e. the measure of how much two variables change together, between the i^{th} and j^{th} elements of a vector can be calculated as expressed in Equation 6.22:

$$COV = SEE^2 \times \left(J' \cdot J\right)^{-1} \qquad \textbf{(6.22)}$$

Where *SEE* is the standard error of estimates, *J* the Jacobian and *J'* the Jacobian matrix transpose (inverses columns and rows). The standard deviation of the diagonal values of the *COV* matrix results in standard deviations for the optimised set of parameters, hence giving measurable confidence criteria for the estimates of the parameters.

Chapter 7
Parametric sensitivity analysis of the Anaerobic Digestion Model No.1

This chapter presents the study on parametric sensitivity analysis carried out on the Anaerobic Digestion Model No.1. The aim of the study was to evaluate the relative influence of the model initial conditions, based on default values, on a desired model output. Results suggest that an accurate assessment of the identified initial conditions can result in more accurate predictions during the start-up phase of an anaerobic digestion system or during the modelling of batch assays. However, the impact of initial conditions on the model prediction reduces with increase in simulation time and ceases after a period corresponding to about two complete hydraulic retention times.

7.1. Introduction

The Anaerobic Digestion Model No.1 (ADM1) is characterised by a complicated structure, including an extensive number of parameters used in differential and algebraic equations. The model requires data for the initial state parameters representing the original state of the reactor, in addition to a complete feedstock characterisation. These two sets of data are used as initial conditions and input influent characteristics respectively for the differential equations involved in the

model. The initial conditions used for the model parameters and representing the characteristics of the inoculum have a major impact on the simulation of the start-up of the digestion process as they correspond to what is in the reactor at time zero. Thereafter, the system should be almost fully described by the characteristics of the feedstock. A set of values for these initial conditions can be obtained from laboratory analysis or from the list of default values collated from the literature. Although the former will ensure greater model accuracy than the latter, it can be significantly more expensive due to the large number of variables involved. To save analytical costs both methods can be combined, but this will involve firstly identifying the relative importance or sensitivity of each parameter to the desired model output, and secondly, allocating greater amount of resources for the accurate determination of the values of the highly sensitive parameters.

Only a limited amount of work has been reported on the screening of the ADM1 initial conditions, since most of the kinetic parameters and stoichiometric coefficients have limited variability (Batstone *et al.* 2002), or often rely on the qualitative sensitivity proposed with the model (Blumensaat and Keller 2005). Whereas some authors have proposed different methodologies for the characterisation of the ADM1 feedstock that involve limited practical measurements (see for instance section 4.2.2), a sensitivity analysis would enable the determination of a subset of relevant initial conditions for the model parameters, which once ranked according to their sensitivity indices could give better insight into the model. Taking into consideration the fact that the main function of the ADM1 is to reproduce as accurately as possible the dynamic variations of an anaerobic digester, there is need for a better understanding of the model performance regarding the sensitivity of the biogas production (i.e. model output) to the model initial conditions. This is

particularly important when the ADM1 is used to simulate batch tests for which the initial conditions are the unique input of the system (Donoso-Bravo *et al.* 2011). Thus, the study reported in this chapter used sensitivity analysis techniques, namely, the Morris method as modified by Campolongo *et al.* (2007) and the Sobol' approach (Sobol' 1993), to evaluate the impact and interactions of the default initial parametric conditions of the ADM1 on the desired model output (i.e. rate of biogas production).

7.2. Methodology

7.2.1. Theoretical background

A clear distinction between local and global sensitivity analysis has to be made at this stage. Local sensitivity analysis consists of changing one parameter value at a time while all others remain constant at their initial stages. In contrast, global sensitivity analysis, which is the subject of this study, involves changing the values of an entire group of parameters with the use of different sampling methods. Global sensitivity analysis enables the establishment of the overall influence of each parameter on a certain output when all factors are varying (Saltelli *et al.* 2004). It also provides valuable information on the interactions between parameters or group of parameters over the output. Furthermore, global techniques are model independent (i.e. valid despite the additivity or linearity of the model), and take into consideration the variation and probability density function (PDF) of the inputs (Saltelli *et al.* 2004). However, they generally involve heavy computational demands (Makler-Pick *et al.* 2011). Screening methods, regression-based methods and variance-based methods are the most widely used techniques in global sensitivity analysis (Confalonieri *et al.* 2010a). The Morris screening method (Morris, 1991) is particularly used for its low computational requirements (Cariboni *et al.* 2007) while the Sobol' (1993) variance-

based technique is a fully quantitative method known to be the most accurate and is often used as a benchmark (Confalonieri *et al.* 2010a).

7.2.2. Morris and Sobol' techniques

Morris (1991) developed the one-factor-at-a-time elementary effects method, which allows determining two sensitivity coefficients μ and σ for each parameter. The coefficient μ assesses the general influence of the input on the output of interest and σ is used to identify non-linear parameters or parameters interacting with others (Campolongo *et al.* 2007). The value of μ is determined by computing a number of incremental ratios at different points of the input space, then taking the average of their values. Each incremental ratio is defined by Equation 7.1:

$$d_i(X) = \frac{y(x_1,...,x_{i-1},x_i + \Delta, x_{i+1},...,x_k) - y(x)}{\Delta} \qquad \textbf{(7.1)}$$

Where $X = (x_1, x_2, x_3, ..., x_k)$ is any selected value in the space of parameters Ω, Δ is a determined multiple of the distance between levels, and y is the model output. μ and σ are respectively the mean and the standard deviation of the finite distribution of elementary effects obtained by sampling different X from Ω. Hence, high values of μ and σ indicate that the parameter under consideration has a major impact and non-linear effect on the output. In this study, the Morris method as modified by Campolongo *et al.* (2007) is used and provides μ^* instead of μ by averaging the absolute values of the incremental ratios. This refined coefficient prevents the occurrence of effects of opposite signs (Saltelli *et al.* 2004).

Variance-based approaches such as Sobol' measure sensitivity by decomposing the variance of the output into terms of increasing dimension referred to

as partial variances. These variances evaluate the contribution of each input to the uncertainty of the model output (Confalonieri *et al.* 2010b) and introduce sensitivity indexes. The first order sensitivity index S_i measures the contribution of the input parameter (main effect) to the output variance. Equation 7.2 below can be used to determine the first order sensitivity index corresponding to a single factor x_i.

$$S_i = \frac{V\left[E(Y / x_i)\right]}{V(Y)} \qquad \textbf{(7.2)}$$

Where $V(Y)$ is the total output variance and $V[E(Y/x_i)]$ represents the variance of the conditional expectation. Higher sensitivity indices can be calculated in the same way, but their estimation is computationally expensive in terms of model simulation time. Homma and Saltelli (1996) consequently introduced the total sensitivity index S_{Ti}, which is the sum of all sensitivity indexes from an input and can be calculated using Equation 7.3:

$$S_{Ti} = \sum S_i + \sum_{j \neq i} S_{ij} + \ldots + S_{1\ldots n} \qquad \textbf{(7.3)}$$

When comparing the results of different sensitivity analysis methods, it is common to focus on the top ranked parameters, while potential disagreements for less influential parameters are of minor importance. Savage scores (Savage 1956) can be used to measure consistency between two sets of ranking and are easily calculated (Campolongo and Saltelli 1997) using Equation 7.4:

$$S_i = \sum_{j=i}^{k} \frac{1}{j} \qquad \textbf{(7.4)}$$

Where i is the rank of the i^{th} order input factor for a sample made of k factors.

7.2.3. Model implementation

The implementation of the ADM1 in Matlab/Simulink carried out by Rosen and Jeppsson (2006) is used. Each unit of the model is represented by a system function (S-function) incorporated in Matlab through Simulink which provides an interactive graphical interface. The value of the biogas flow rate normalised to atmospheric pressure (Q_{gas}) is the model output considered in this study, since it represents biogas production from the digester. The production of biogas is considered after the solver first calculation steps, to allow reasonable simulation time. Some parameters were excluded from the analysis, for example those parameters that are user defined such as the operational temperature and the digester physical characteristics and their dependent parameters (e.g. Henry's law coefficients) or fixed carbon content of components. Hence, parameters dependent on temperature, acid-base equilibrium coefficients and acid-base kinetic parameters were kept constant. Only the initial conditions corresponding to the reactor inoculum characteristics at time zero are considered. The reactor system modelled in the study consisted of a one-stage 3700 m^3 capacity (with effective volume of 3400 m^3) completely stirred reactor treating sewage sludge at mesophilic temperatures and operating at a 20 days hydraulic retention time (HRT). Table 7.1 shows the initial model parameters and the range of values used for the study. Due to a wide variation of values found in literature, the parametric values were mainly defined as ranging from half to twice the nominal values used in the original model.

Table 7.1. ADM1 parameters and values for sensitivity analysis (T: triangular, U: uniform).

Variables	Range	Units	PDF	Variables	Range	Units	PDF
Yield of products on substrates				*Yield of biomass on substrates*			
$f_{ac,aa}$	0.2-0.8	kgCOD/kgCOD	U	Y_{aa}	0.04-0.16	$kgCOD_X/kgCOD_S$	U
$f_{ac,su}$	0.205-0.82	kgCOD/kgCOD	U	Y_{ac}	0.03-0.1	$kgCOD_X/kgCOD_S$	U
$f_{bu,aa}$	0.13-0.52	kgCOD/kgCOD	U	Y_{c4}	0.03-0.12	$kgCOD_X/kgCOD_S$	U
$f_{bu,su}$	0.065-0.26	kgCOD/kgCOD	U	Y_{fa}	0.03-0.12	$kgCOD_X/kgCOD_S$	U
$f_{ch,xc}$	0.1-0.4	kgCOD/kgCOD	U	Y_{h2}	3E-02 - 1.2E-01	$kgCOD_X/kgCOD_S$	U
$f_{fa,li}$	0.475-1.9	kgCOD/kgCOD	U	Y_{pro}	0.02-0.08	$kgCOD_X/kgCOD_S$	U
$f_{h2,aa}$	0.03-0.12	kgCOD/kgCOD	U	Y_{su}	0.05-0.2	$kgCOD_X/kgCOD_S$	U
$f_{h2,su}$	0.095-0.38	kgCOD/kgCOD	U	*First order decay rates*			
$f_{li,xc}$	0.015-0.6	kgCOD/kgCOD	U	$K_{dec,Xaa}$	0.01-0.04	d^{-1}	U
$f_{pro,su}$	0.135-0.54	kgCOD/kgCOD	U	$K_{dec,Xac}$	0.01-0.04	d^{-1}	U
$f_{pr,xc}$	0.1-0.4	kgCOD/kgCOD	U	$K_{dec,Xc4}$	0.01-0.04	d^{-1}	U
$f_{sI,xc}$	0.1-0.4	kgCOD/kgCOD	U	$K_{dec,Xfa}$	0.01-0.04	d^{-1}	U
$f_{va,aa}$	0.115-0.46	kgCOD/kgCOD	U	$K_{dec,Xh2}$	0.01-0.04	d^{-1}	U
$f_{xI,xc}$	0.1-0.4	kgCOD/kgCOD	U	$K_{dec,Xpro}$	0.01-0.04	d^{-1}	U
$f_{pro,aa}$	0.025-0.1	kgCOD/kgCOD	U	$K_{dec,Xsu}$	0.01-0.04	d^{-1}	U
First order parameters				*Monod maximum specific uptake rates*			
K_{dis}	0.25-1	d^{-1}	T[a]	$K_{m,aa}$	25-100	d^{-1}	T[a]
$K_{hyd,ch}$	5-20	d^{-1}	T[a]	$K_{m,ac}$	4-16	d^{-1}	T[a]
$K_{hyd,li}$	5-20	d^{-1}	T[a]	$K_{m,c4}$	10-40	d^{-1}	T[a]
$K_{hyd,pr}$	5-20	d^{-1}	T[a]	$K_{m,fa}$	3-12	d^{-1}	T[a]
50% inhibitory concentrations				$K_{m,h2}$	17.5-70	d^{-1}	T[a]
$K_{Ih2,c4}$	5E-06 - 2E-05	$kgCOD/m^3$	U	$K_{m,pro}$	6.5-26	d^{-1}	T[a]
$K_{Ih2,fa}$	2.50E-06 - 1E-05	$kgCOD/m^3$	U	$K_{m,su}$	15-60	d^{-1}	T[a]
$K_{Ih2,pro}$	1.75E-06-7E-06	$kgCOD/m^3$	U	*Half saturation values*			
$K_{Inh3,ac}$	9E-04 - 3.6E-03	M	U	$K_{s,aa}$	0.15-0.6	$kgCOD/m^3$	U
Nitrogen content of components				$K_{s,ac}$	0.08-0.3	$kgCOD/m^3$	U
N_{aa}	0.0035-0.014	kmoleN/kgCOD	U	$K_{s,c4}$	0.1-0.4	$kgCOD/m^3$	U
N_{bac}	0.0029-0.0114	kmoleN/kgCOD	U	$K_{s,fa}$	0.2-0.8	$kgCOD/m^3$	U
N_I	0.0021-0.0086	kmoleN/kgCOD	U	$K_{s,h2}$	3.5E-06 - 1.4E-05	$kgCOD/m^3$	U
N_{Xc}	0.0014-0.0054	kmoleN/kgCOD	U	$K_{s,pro}$	0.05-0.2	$kgCOD/m^3$	U
				$K_{s,su}$	0.25-1	$kgCOD/m^3$	U
				$K_{s,IN}$	5E-05 - 2E-04	$kgCOD/m^3$	U
				K_La	100-400	d^{-1}	T[a]
Initial values for soluble components[b]							
S_{cat}	0.02-0.08	M	U	S_{su}	0.006-0.024	$kgCOD/m^3$	U
S_{an}	0.01-0.04	M	U	S_{aa}	0.00265-0.0106	$kgCOD/m^3$	U
S_{hva}	0.0055-0.022	$kgCOD/m^3$	U	S_{fa}	0.0495-0.198	$kgCOD/m^3$	U
S_{hbu}	0.0065-0.026	$kgCOD/m^3$	U	S_{va}	0.006-0.024	$kgCOD/m^3$	U
S_{hpro}	0.008-0.032	$kgCOD/m^3$	U	S_{bu}	0.0065-0.026	$kgCOD/m^3$	U
S_{hac}	0.1-0.4	$kgCOD/m^3$	U	S_{pro}	0.008-0.032	$kgCOD/m^3$	U
S_{hco3}	0.07-0.28	$kgCOD/m^3$	U	S_{ac}	0.1-0.4	$kgCOD/m^3$	U
S_{nh3}	0.00205-0.0082	$kgCOD/m^3$	U	S_{h2}	1.15E-07-4.6E-07	$kgCOD/m^3$	U
S_{gas_h2}	5.1E-06-2.04E-05	$kgCOD/m^3$	U	S_{ch4}	0.0275-0.11	$kgCOD/m^3$	U
S_{gas_ch4}	0.815-3.26	$kgCOD/m^3$	U	S_{IC}	0.075-0.3	M	U
S_{gas_co2}	0.007-0.028	$kgCOD/m^3$	U	S_{IN}	0.065-0.26	M	U
				S_I	0.165-0.66	$kgCOD/m^3$	U
Initial values for particulate components[b]							
X_{xc}	0.155-0.62	$kgCOD/m^3$	U	X_{fa}	0.12-0.48	$kgCOD/m^3$	U
X_{ch}	0.014-0.056	$kgCOD/m^3$	U	X_{c4}	0.215-0.86	$kgCOD/m^3$	U
X_{pr}	0.05-0.2	$kgCOD/m^3$	U	X_{pro}	0.07-0.28	$kgCOD/m^3$	U
X_{li}	0.0145-0.058	$kgCOD/m^3$	U	X_{ac}	0.38-1.72	$kgCOD/m^3$	U
X_{su}	0.21-0.84	$kgCOD/m^3$	U	X_{h2}	0.16-0.64	$kgCOD/m^3$	U
X_{aa}	0.59-2.36	$kgCOD/m^3$	U	X_I	12.8-51.2	$kgCOD/m^3$	U

[a]Recommended by Benedetti et al. 2008, [b]Values according Rosen and Jeppsson 2006

The parameters were considered having different distribution functions (PDF) with a uniform distribution for most (Campolongo and Saltelli 1997) or with a triangular distribution when further information was available (Benedetti *et al.* 2008). The analysis was run using SimLab developed by the Joint Research Centre of Ispra (Ispra, Italy). It is composed of different modules with a statistical pre-processor to generate samples, model execution and a statistical post-processor to estimate uncertainty and sensitivity (SimLab 2009). SimLab functions can be used directly in the Matlab environment when a set of parameters is created and the model to be used is specified. However, the ADM1 has been run in Simulink and it was necessary to adapt an interface between the different environments as shown in Figure 7.1.

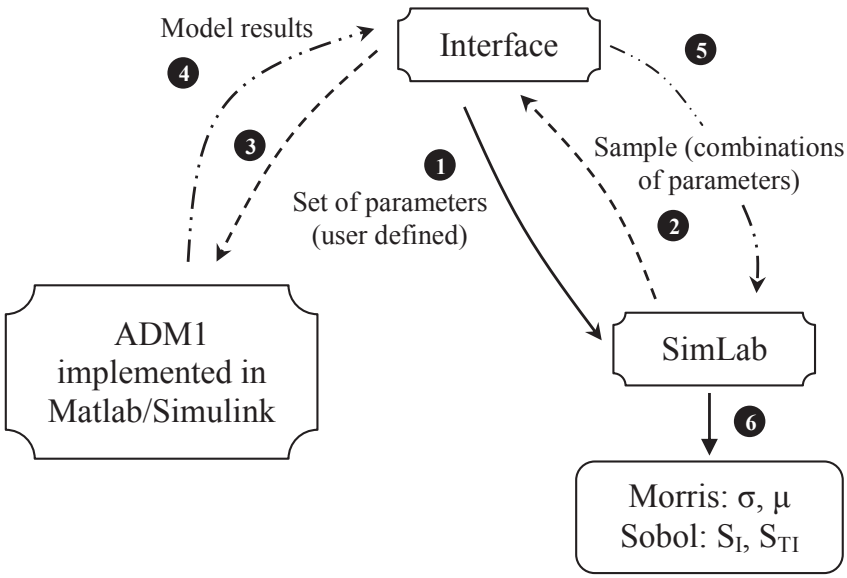

Figure 7.1. Interface between the different steps of sensitivity analysis.

The first step involved defining the complete set of parameters to be studied with their range and distribution (❶). SimLab then generated a sample which consisted of a

combination of different parameters (❷) that could be used as model inputs in Matlab/Simulink (❸). After simulation, the model outputs (❹) were stored and transformed (❺) and used to perform a sensitivity analysis on the chosen parameters (❻). The range of variation selected for the parameters' initial conditions can impact on the sensitivity analysis results and different solutions are reported in the literature. Morris (1991) scaled inputs so that they ranged from 90% to 110% of a nominal value while Campolongo and Saltelli (1997) assumed the parameters to vary from half to double their nominal value. In the ADM1, literature shows that parameters such as particulate fractions are often varying between extreme values when the model is applied to different substrates. Moreover, values for hydrolysis rates presented in the original model are now considered to be at least ten times too large (Zaher *et al.* 2009b). Consequently, the largest range of variation has been adopted in this study for both Morris and Sobol' analysis. The parameters chosen for the influent feedstock have been set in order to allow all internal modes of the model to be activated (Rosen and Jeppsson 2006) and the feedstock characterisation is relevant for substrates mainly containing soluble organic compounds, *viz.* for which disintegration and hydrolysis are not the limiting steps. The use of an algorithm adapted to stiff problems is essential and good results were obtained with the variable-step solver *ode23t*. In order to reduce stiffness problems and computational efforts, the implementation chosen for the model was DAE2 (differential algebraic equation) fitted with a pH and S_{h2} solver. With the use of an appropriate algorithm the analysis could be conducted over the first calculations steps. Since the use of an implicit solver can lead to slightly different results under the same simulated conditions, the calculations were repeated in triplicate with varying absolute and relative tolerances to ascertain that the sensitivity results were coherent. The results from the analysis were evaluated to

estimate the importance of each initial condition on the simulated biogas production. With over 80 parameters, a sensitivity method requiring low computational costs such as Morris was found useful for an initial assessment of the parametric sensitivity. It was then possible to use the Sobol' analysis on a subset of the 20 most influential inputs to compare their ranking. Only 20 parameters were considered during the Sobol' analysis in order to limit computational costs. Considering the number of parameters included in the study, the total number of simulations carried out required 5632 and 930 model runs with Sobol' and Morris techniques respectively.

7.3. Results and discussion

7.3.1. Identified parameters

Values for μ^* of Morris and the total effect indices of Sobol' for the ten most important parameters along with Savage scores are shown in Table 7.2. The table introduces the results of the 10 most important parameters exclusively since both Sobol' and Morris methods indicated that the impact of the remaining parameters was not substantial. From the results of the Sobol' total effects measure, the ten parameters whose initial conditions have the highest impact on Q_{gas} are the soluble inorganic carbon concentration (S_{IC}), soluble inorganic nitrogen concentration (S_{IN}), concentration of cations (S_{CAT}), yield of acetate from amino-acid degradation ($f_{ac,aa}$), yield of butyrate from amino-acid degradation ($f_{bu,aa}$), yield of valerate from amino-acid degradation ($f_{va,aa}$), protein hydrolysis first order constant ($K_{hyd,pr}$), nitrogen content of amino acids (N_{aa}), nitrogen content of biomass (N_{bac}) and soluble methane ($S_{gas,ch4}$). These results are identical in all three simulations for the top three parameters, with S_{IC} being the parameter with the highest Sobol' total index and S_{IN} always ranking second. For the Morris μ* analysis, S_{IC} is ranked first in two out of

three simulations (and ranking second in the third simulation) and S_{IN} comes second in two out of three cases (and first in the third simulation), S_{CAT} can be considered as the third most sensitive parameter on average.

Table 7.2. Morris μ^*, Sobol' S_{Ti} and Savage scores for the top ten parameters.

Simulation n°	Parameter initial condition	Morris μ^*	Sobol' S_{Ti}	Morris ranks	Sobol' ranks	Morris Savage scores	Sobol' Savage scores
1	$f_{ac,aa}$	11356.17	0.05603403	5	5	0.8456	0.8456
	$f_{bu,aa}$	9711.57	0.02157784	6	6	0.6456	0.6456
	$f_{va,aa}$	6471.88	0.01844803	9	7	0.2111	0.4790
	$K_{hyd,pr}$	6290.03	0.00916628	10	9	0.1000	0.2111
	N_{bac}	8031.21	0.00450073	8	10	0.3361	0.1000
	S_{IC}	69025.0357	0.63362994	1	1	2.9290	2.9290
	S_{IN}	67719.8265	0.57531421	2	2	1.9290	1.9290
	S_{cat}	28090.6741	0.08393106	3	3	1.4290	1.4290
	N_{aa}	9537.69	0.07363917	7	4	0.4790	1.0956
	S_{gas_ch4}	14466.5392	0.01115434	4	8	1.0956	0.3361
2	$f_{ac,aa}$	12658.22	0.05663744	4	5	1.0956	0.8456
	$f_{bu,aa}$	9374.20	0.0198736	6	6	0.6456	0.6456
	$f_{va,aa}$	7183.55	0.01895357	9	7	0.2111	0.4790
	$K_{hyd,pr}$	5934.69	0.00942479	10	9	0.1000	0.2111
	N_{bac}	8118.41	0.00531267	8	10	0.3361	0.1000
	S_{IC}	69319.8411	0.63303684	1	1	2.9290	2.9290
	S_{IN}	68232.4651	0.58193393	2	2	1.9290	1.9290
	S_{cat}	26369.9462	0.0844534	3	3	1.4290	1.4290
	N_{aa}	9129.23	0.07363917	7	4	0.4790	1.0956
	S_{gas_ch4}	12074.371	0.01113246	5	8	0.8456	0.3361
3	$f_{ac,aa}$	11533.68	0.05714219	5	5	0.8456	0.8456
	$f_{bu,aa}$	8849.48	0.02217603	8	6	0.3361	0.6456
	$f_{va,aa}$	7242.46	0.01992179	9	7	0.2111	0.4790
	$K_{hyd,pr}$	6096.6	0.00863572	10	9	0.1000	0.2111
	N_{bac}	10552.82	0.00551739	7	10	0.4790	0.1000
	S_{IC}	69089.959	0.63503056	2	1	1.9290	2.9290
	S_{IN}	69338.0673	0.58778436	1	2	2.9290	1.9290
	S_{cat}	27033.7802	0.0882904	3	3	1.4290	1.4290
	N_{aa}	10926.99	0.07833537	6	4	0.6456	1.0956
	S_{gas_ch4}	14005.6159	0.00967233	4	8	1.0956	0.3361

Overall, a good consistency was found between the different analyses for the ten first parameters and both Sobol' and Morris techniques gave similar results in terms of

parameter identification as shown on Figure 7.2 in which results from the Morris analysis are plotted against the Sobol' total sensitivity index.

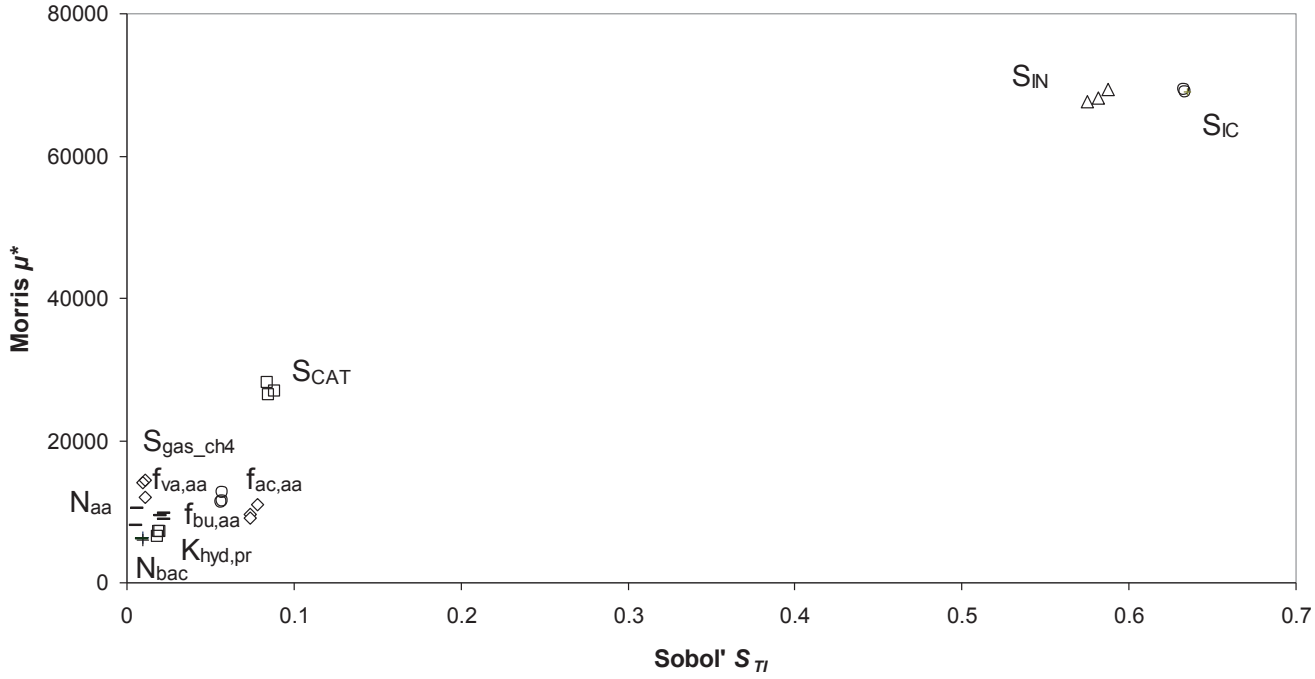

Figure 7.2. Comparison between Morris μ^* and Sobol' total order effect for the ten most influential ADM1 parameters.

Figure 7.3 presents the values of μ^* and σ for the ADM1 parameters as usually displayed. The figure shows that none of the parameters is located at the lower right hand side of the graph (i.e. high μ^* and low σ), meaning that all the parameters have less significant first-order effect. However, the three most sensitive parameters (S_{IC}, S_{IN} and S_{CAT}) have a high impact because of their main effect and interactions with other parameters. S_{IN} and S_{IC} show the highest interactions with other parameters.

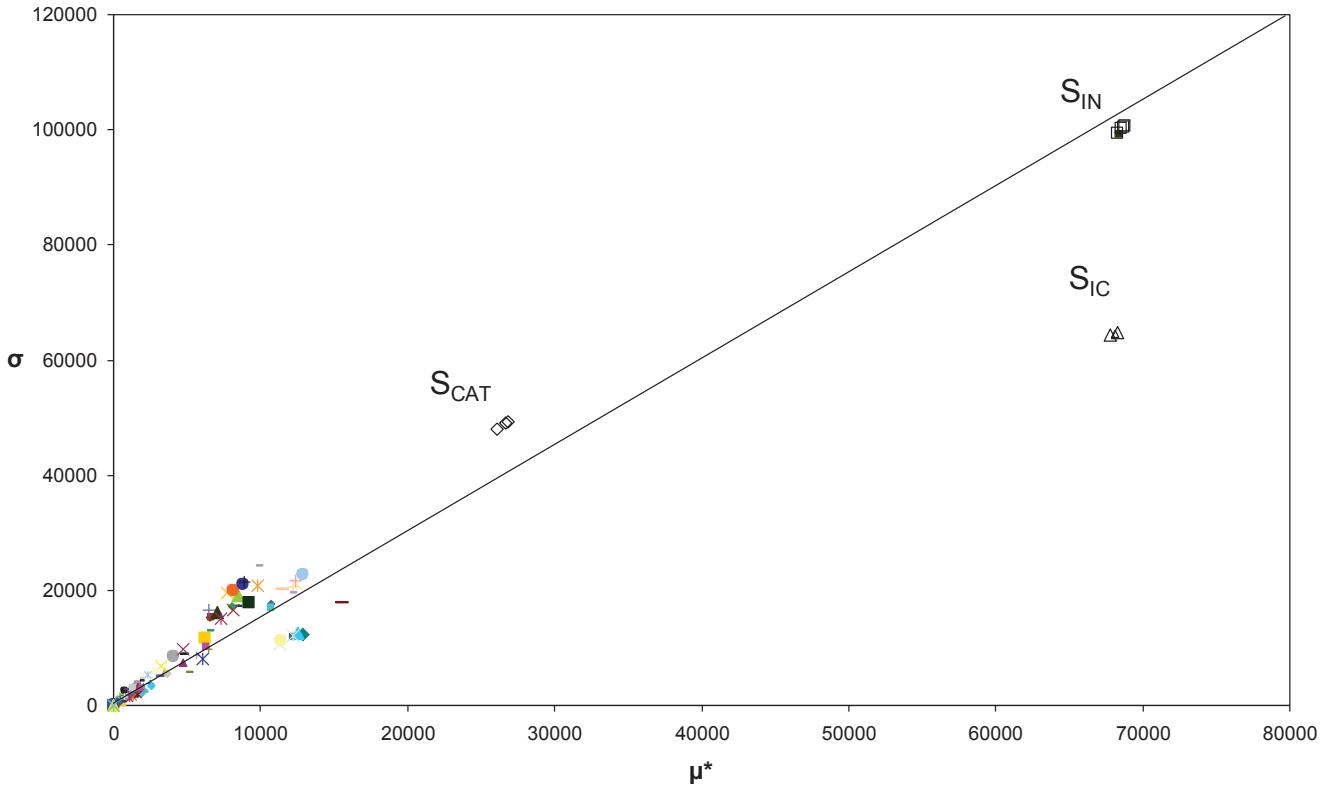

Figure 7.3. Morris sensitivity analysis: scatterplot of σ versus μ^*.

Figure 7.4 shows the effect of a simultaneous variation of S_{IN} and S_{IC} on the biogas flow rate for the anaerobic digestion system considered in this study. Simulation was carried out over a 100 days. The effects of S_{IN} and S_{IC} initial conditions clearly have a major impact for the first 20 days of simulation (Figure 7.4a), which is equivalent to the hydraulic retention time of the reactor system considered in the study. From day 20 to 40, the effects of inorganic carbon and nitrogen initial conditions decrease rapidly and almost have no impact from day 40 to the end of simulation. The simulated biogas production over 100 days hence shows that a variation in the initial conditions of S_{IN} and S_{IC} can impact on the output of the model for a period of almost 40 days representing two complete HRT. This period also corresponds to the start-up period of a standard anaerobic digestion system.

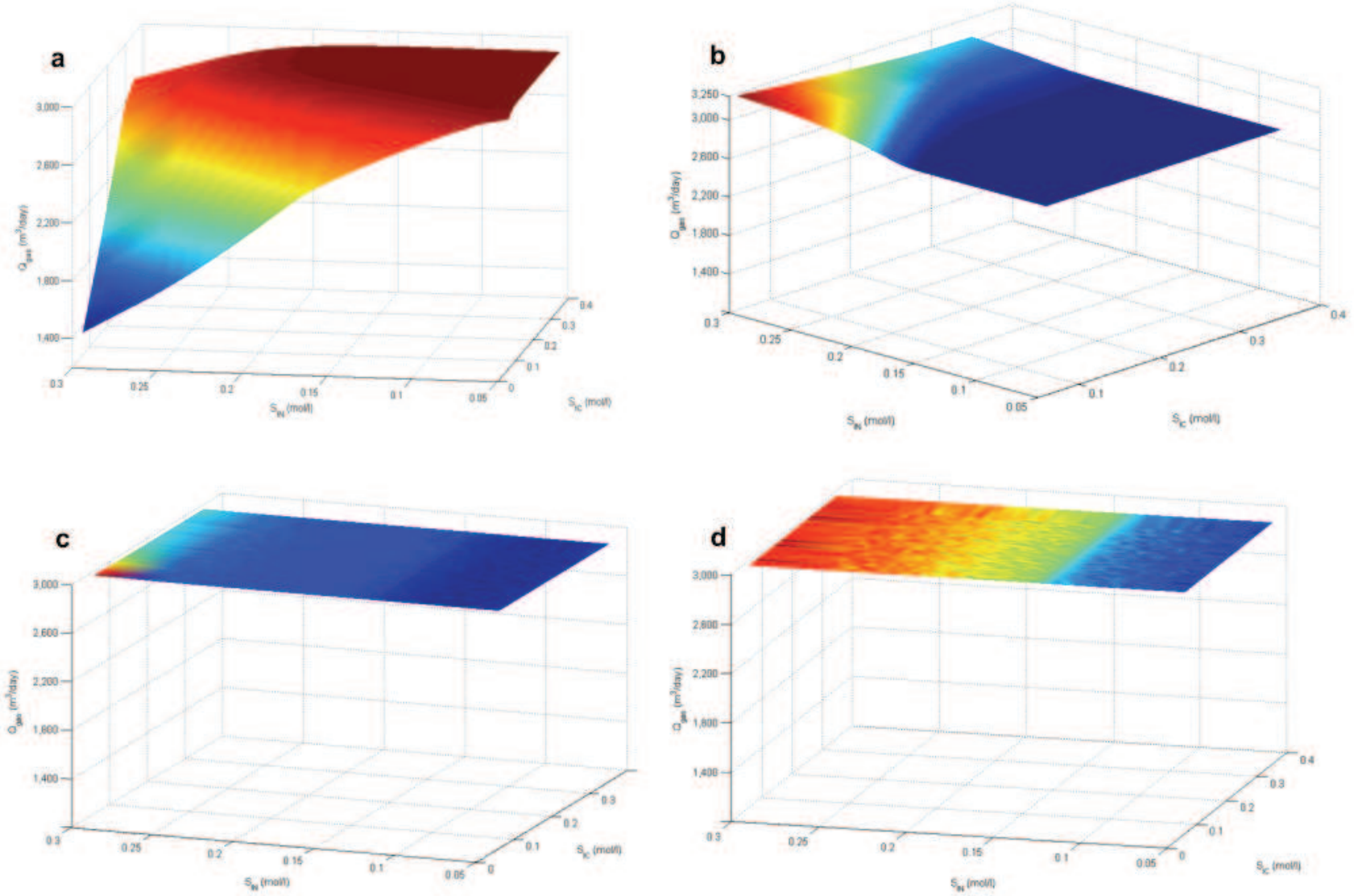

Figure 7.4. Effect of initial values of S_{IC} and S_{IN} simultaneous variation on Q_{gas}: (a) Days 0 to 20, (b) Days 20 to 40, (c) Days 40 to 60, (d) Days 60 to 100. The colour scheme used on the three dimensional plots has no significance on the interpretation of the figure.

The initial concentration of inorganic nitrogen particularly impacts on the predicted biogas production for almost 40 days while a simultaneous variation of S_{IN} and S_{IC} appears to have the same effect on the model predictions.

7.3.2. Practical significance

Parametric sensitivity analysis of the ADM1 model with respect to biogas production, using both the Morris and Sobol' methods, has shown that initial conditions of S_{IC} and S_{IN} are the most influential on the output uncertainty. The variation of the initial concentrations of inorganic carbon and inorganic nitrogen, present in the original seed inoculum, can have an impact on the biogas flow rate during start-up, lasting up to an equivalent of two complete hydraulic retention cycles. Thereafter, the system performance will be determined mainly by the influent feedstock characteristics even if it is suggested to wait up to 50 HRT to obtain the numerical stabilisation of the system when running the ADM1 for benchmarking. The study also suggests that S_{IC} and S_{IN} are more sensitive to biogas yield through their indirect interaction with other parameters associated with digestion processes. This is not surprising since both parameters are also by-products of the breakdown of carbonaceous and proteinous substrates in the reactor. Thus, S_{IC} and S_{IN} provide an estimate of the amount of biodegradable matter originally found in the system. The methane yield of a specific organic feedstock is often related to its chemical organic demand (COD) when modelling anaerobic digestion (Ward *et al.* 2008; Martin Santos *et al.* 2010), since most of the models use COD to describe organic substrate. The drawback in relating COD directly to potential biogas production is the risk of exaggerating the methane potential for substrates containing significant amounts of non-biodegradable or non-readily biodegradable COD. A more detailed characterisation of the substrate in terms

of carbon and nitrogen content as found in the ADM1 should thus provide more accurate model predictions. It is important to note that S_{IC} is the sum of soluble carbon dioxide and bicarbonate concentrations, thus its value at a given temperature will be dependent on the pH of the digesting culture. S_{IN} is the sum of ammonium and ammonia concentrations, and its value will be important in anaerobic systems treating nitrogen deficient substrates. Since ammonium and ammonia are by-products of amino acid degradation, the amount of S_{IN} in a digester can provide an indication of the proteinaceous nature of the digesting substrate in addition to the potential inhibition due to excessive ammonia concentrations. The nitrogen content of both amino acids and biomass identified by the sensitivity analysis are also relevant to assess nitrogen limitation for biomass growth as explained in section 2.2.2. In the model, S_{CAT} represents metallic ions and strong bases. It can be treated as an inert compound with no consumption or reaction terms (Batstone *et al.* 2002). Common cations can have a major effect on anaerobic digestion and can either stabilise the process or be inhibitory at high levels as seen in section 2.3.2 and further emphasised in section 3.3.2. Feng *et al.* (2006) reported that S_{CAT} strongly influences pH due to the charge balance (see Equation 4.7), thus impacting on the entire digestion process. Other parameters identified, namely $f_{ac,aa}$, $f_{bu,aa}$, $f_{va,aa}$ and $K_{hyd,pr}$, refer to products from amino acid or hydrolysis of proteins to amino acids since the latter is directly converted to acetate. This acetate will subsequently be converted to methane and hence impact directly on the biogas production. The initial value of $S_{gas,ch4}$ influences the calculation of gas phase equations (see Equation 4.8) and therefore the gas flow rate.

7.4. Conclusions

In this study, both Sobol' and Morris sensitivity methods have identified initial values of soluble inorganic carbon (S_{IC}), soluble inorganic nitrogen (S_{IN}) and cation concentration (S_{CAT}) as the most influencing on the performance of an anaerobic digester during start-up; the values of these initial conditions being dependent on the source of the seed inoculum and found in the system at time zero. It has been found that the estimation of these parameters' initial conditions, together with the complete digested feedstock characterisation, are likely to provide more accurate results on the model prediction particularly when the ADM1 is used to simulate batch assays. It has also been shown that the effect of the initial digester conditions decreases with time, and almost completely ceases after a period corresponding to about twice the effective hydraulic retention time, which is within the range of start-up periods of a standard completely mixed anaerobic digester, without recirculation. Thereafter, model results will be dependent mainly on the characteristics of the raw feedstock being treated. Hence, the careful measurement of the parameters identified in this study is of crucial importance to obtain accurate and reliable model predictions during the start-up phase of the anaerobic system being modelled. Strong interactions have been highlighted between the ADM1 parameters, thereby emphasising the importance of global sensitivity analysis for the study of complex models. This work has also shown that both Morris and Sobol' methods can be used for sensitivity analysis of highly non-linear models, although the Sobol' method requires greater amount of computational time. For the analysis of complex models such as the ADM1, which involves many parameters, less computing time can be achieved by firstly using the Morris method as a preliminary screening technique and later applying the Sobol' method.

Chapter 8
Investigating the effects of sodium ions on the anaerobic digestion process

This chapter presents the study carried out to determine the inhibitory effects of varying concentrations of sodium ions on the anaerobic digestion process. A combination of experimental and modelling approaches has been employed, including the modification of the ADM1 through the addition of a non-competitive inhibition function that considers the effect of sodium on acetoclastic methanogens and its consequent impact on biogas production and composition. Experimental studies consisted of both batch and reactor tests designed to obtain quantitative values of key parameters for model calibration and validation. The calibrated model was used to reproduce experimental observations and the projected effect of ammonia nitrogen on sodium toxicity. Results suggest that the adjustment of a reduced set of parameters and limited experimental work can lead to accurate simulation for pH, VFA, biogas production and composition.

8.1. Introduction

Sodium is a chemical element widely found in water bodies and everyday life products. It is a crucial compound in both human and animal life because of its regulating effect on body fluid volumes. Although sodium is detected in most of

biodegradable substrates, its usually low concentration rarely affects microorganisms involved in anaerobic digestion. In most cases, the impact of sodium in anaerobic digesters treating municipal wastewater and the biodegradable fraction of municipal waste seems to be minor and has not been found detrimental. However, toxic levels of sodium are common in systems treating wastewater from food processing industries (Soto *et al.* 1993; Feijoo *et al.* 1995; Gebauer 2004), chemical industries or reactors using macroalgae as a substrate (see Chapter 3.3.2). High salts content, not only from sodium, but also potassium, calcium and magnesium ions, exists in macroalgae and has been reported to be inhibitory to non-adapted microorganisms. In this work, the impact of sodium concentration on acetoclastic methanogens is studied using a combination of experimental and modelling approaches. The ADM1 is used as a platform for process simulation and has been modified to include an extra inhibition function that considers the effect of sodium on acetoclastic methanogens. Batch tests and reactor studies were conducted and results used to calibrate the modified version of the ADM1. Linear regression analyses are used to assess the suitability of the measured parameters for process monitoring.

8.2. Methodology

8.2.1. Batch and reactor studies

Batch assays were conducted according to the methodology introduced in Chapter 6.1.2. The assays were conducted in triplicate during 40 days to determine digestion kinetics. The method was adapted from Hansen *et al.* (2004) and bottles were inoculated with 400 ml of anaerobically digested sludge (16 g/l TS) and 100 ml non-growth medium adapted from Akunna *et al.* (1993) (2.7 g/l KH_2PO_4, 3.5 g/l K_2HPO_4, 0.005 g/l $MgSO_4.7H_2O$, 0.0005 g/l $CaCl_2$, 0.0005 g/l $FeCl_2$, 0.0005 g/l KCl_2, 0.0001

g/l $CoCl_2.6H_2O$, 0.0001 g/l $NiCl_2$). Solutions containing 10 grams of green peas prepared according to the procedure presented in Chapter 6.4 and diluted with 100 ml of tapwater were used. Blanks containing only inoculum, medium and tapwater were also prepared to consider the effect of the inoculum activity on biogas production.

The anaerobic reactor described in Chapter 6.1.1 was used to assess the effect of increasing concentration and accumulation of sodium within the system. The reactor was firstly inoculated with anaerobically digested sludge and set in batch mode until the start-up of biogas production. Feeding was carried out once daily with 100 g of blended fresh peas (*Pisum sativum*) diluted with 200 ml of tapwater. The reactor was operated under mesophilic temperatures (37°C±1°C) with a 20 days hydraulic retention time. At steady state, varying sodium quantities in the form of sodium bicarbonate ($NaHCO_3$) were introduced into the reactor at specific intervals, as shown in Table 8.1. The addition of sodium as $NaHCO_3$ ensured that the pH values of the digesting culture were maintained at a suitable range during the experiment.

Table 8.1. Summary of reactor additions.

Day	Amount $NaHCO_3$ added (g)	Equivalent sodium in reactor (mol/l)	Method of addition
0-27	-	-	No addition
28	35	0.083	Added once, directly in the reactor
29-34	-	-	No addition
35	35	0.14	Added once, directly in the reactor
36-41	-	-	No addition
42	35	0.2	Added once, directly in the reactor
43-48	-	-	No addition
49	35	0.26	Added once, directly in the reactor
50-55	-	-	No addition

8.2.2. Model implementation

The implementation of the ADM1 in Matlab/Simulink carried out by Rosen and Jeppsson (2006) is used. Non-competitive inhibitions from hydrogen and free

ammonia are originally implemented in the ADM1 along with pH inhibition. Since sodium has been reported to lower the maximum specific growth rate and yield of acetoclastic methanogens (Rinzema *et al.* 1987), an extra inhibition factor I_{Na^+} can be applied to the rate of acetate uptake as expressed in Equation 8.1:

$$I_{acetate} = I_{pH,ac} \cdot I_{IN,\lim} \cdot I_{Nh3} \cdot I_{Na^+} \qquad \textbf{(8.1)}$$

Where $I_{acetate}$ is the overall inhibition function applied to the rate of acetate uptake, $I_{pH,ac}$ is the pH-inhibition function, $I_{IN,lim}$ is the inorganic nitrogen limitation inhibition function, I_{Nh3} is the ammonia nitrogen inhibition function and I_{Na^+} is a non-competitive function taking into consideration the effect of sodium concentration not represented in the original model. I_{Na^+} can be expressed as shown in Equation 8.2:

$$I_{Na^+} = \frac{1}{1 + \frac{S_{Na^+}}{K_{I,Na^+}}} \qquad \textbf{(8.2)}$$

Where K_{I,Na^+} is the inhibitory sodium concentration for acetate degrading organisms and S_{Na^+} is the concentration of sodium implemented within the model as expressed in Equation 8.3:

$$\frac{dS_{Na^+}}{dt} = \frac{q_{in}}{V_{liq}}\left(S_{Na^+,in} - S_{Na^+}\right) \qquad \textbf{(8.3)}$$

Where q_{in} is the reactor inflow, V_{liq} the effective volume of the reactor. S_{Na^+} and S_{Na,in^+} are the initial concentrations of sodium in the system and in the feedstock respectively. An adaptation phenomenon to highly saline environments for microorganisms involved in anaerobic degradation has been observed by many

authors (Bashir and Matin 2004a; Gebauer 2004; Sialve *et al.* 2009) and is believed to occur through the adaptation of the original microorganisms towards tolerance to higher osmotic pressure induced by sodium, or a complete shift in microbial population, leading to the replacement of the original population by a subsequent saline-tolerant population. The experimental design and duration adopted in this study was to ensure negligible adaptation of the methanogenic archaea to sodium.

8.2.3. Linear regression analysis

Statistical analyses were performed using the SPSS 18.0 package (SPSS International, Chicago, IL), one-way ANOVA was used to assess significance of factors on response variables and Pearson correlation was used to determine the relationship between quantitative variables. All statistical analyses were preceded by the determination of model assumptions. Appropriate experimental design was used to ensure independence of data, equality of variances and normal distribution were verified by Levene's and Shapiro-Wilk tests respectively.

8.3. Results and discussion

8.3.1. Experimental results and parameters estimation

Figure 8.1 shows the cumulative methane production obtained with green peas net of blank results. The ultimate methane yield is found equal to 0.19 litres CH_4/gVS_{added} with a corresponding disintegration constant equal to 0.093 day^{-1} according to the methodology introduced in Chapter 6.3.6. The methane production rate constant was also determined using Equation 6.17 (first-order rate) and Equation 6.18 (least-squares). It can be seen from the figure that the curve determined by using the least-

square method fits experimental results well and the value of the disintegration constant was consequently adjusted to 0.054 day^{-1}.

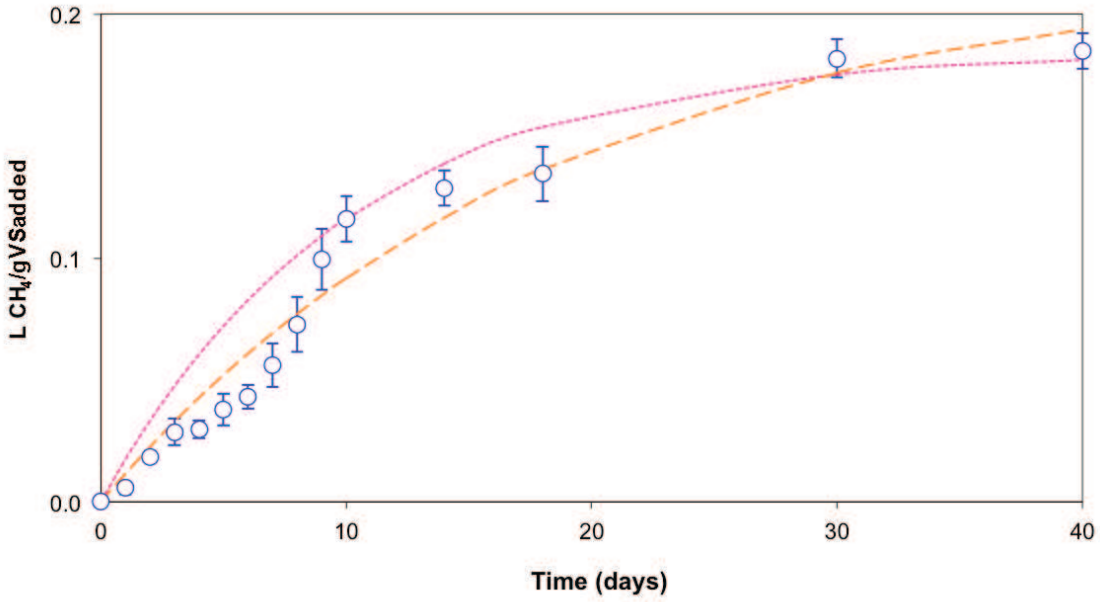

Figure 8.1. Cumulative methane production from green peas: experimental data (○), estimation from first-degree equation (—) and estimation by the method of least-squares (---). Error bars represent the standard deviation between triplicate experiments.

The values of soluble variables such as chemical oxygen demand and volatile fatty acids (VFA) were derived from experimental data obtained from the reactor studies. The concentration of inorganic carbon in the effluent (S_{IC}) was obtained from partial alkalinity, i.e. addition of acid to the sample until pH reached 5.75, since it is believed that inorganic carbon will be mainly due to bicarbonate between pH 6 and 8 (Van Haandel and Lettinga 1994). Total metallic cation concentration (S_{CAT}) was estimated by the total alkalinity of the digested substrate. The concentration of inorganic nitrogen (S_{IN}) was estimated by the measurement of ammonium nitrogen concentration. The three aforementioned parameters were also measured in the anaerobically digested sludge used for reactor inoculation. Their respective values were used as initial conditions for the model equations (see section 7.3.2). VFA

components (S_{va}, S_{bu}, S_{pro} and S_{ac}) were calculated from the total VFA and apportioned according to the proportion of each component in the effluent. First order parameters corresponding to the hydrolysis kinetic rates of carbohydrates, lipids and proteins were set at rates similar to the disintegration step since their influence is not significant for homogenous substrates (Blumensaat and Keller 2005; Feng *et al.* 2006). Parameters representing yields of products on substrates were calculated from the concentrations of protein, carbohydrate and fibre. For example, cellulose content was estimated from the content of crude fibre according to Matrone *et al.* (1946) and lignin content from the difference between acid detergent fibre and cellulose (Reichert and MacKenzie 1982). Setting the yield of soluble inert to zero, $f_{pr,xc}$, $f_{ch,xc}$, $f_{li,xc}$ and $f_{xi,xc}$ were calculated to 0.189, 0.263, 0.011 and 0.536 kgCOD.kgCOD^{-1} respectively. Influent inert particulates were estimated from the total solids and volatile solids values. $K_{I,Na}^{+}$ was adjusted to 0.21 mol/l by fitting the model outputs to the experimental data, mainly VFA and biogas composition. Similarly, maximum specific uptake rate and half saturation values were adjusted for acetate from experimental data. Chen *et al.* (2008) reported IC_{50} values for sodium inhibition ranging from 0.24 to 2.3 mol/l, depending on reactor configuration, substrate, potential microbial adaptation and presence of other cations. The relatively small value obtained in this study is believed to be characteristic of the particular system considered with no biomass adaptation or significant concentration of other cations. The values of the operating parameters, i.e. temperature, flow rate and reactor size were also added in the model. Other parameters such as particulate components, half saturation values or decay rates were taken to be equal to the values suggested by Batstone *et al.* (2002) and from the Matlab implementation of Rosen *et al.* (2006). Experimental results from the reactor study are presented along with model outputs in section 8.3.2.

8.3.2. Modelling of reactor performance

The ability of the model to simulate appropriately the effects of sodium addition in the reactor was evaluated by comparing experimental values with simulation results. Table 8.2 summarises the characteristics of the reactor effluent over the experimental period.

Table 8.2. Effluent characteristics and biogas production.

Parameters	Minimum	Maximum	Average	Standard deviation	N. of samples
pH	6.8	7.6	7.3	0.2	57
COD_S (mg COD/l)	1455.0	19064.0	5741.3	5637.4	18
COD_T (mg COD/l)	13128.0	29313.0	18988.0	8968.9	3
TS (g/l)	8.1	19.2	12.7	3.4	14
VS (%TS)	43.3	76.0	59.7	10.6	14
VFA (mg COD/l)	304.9	7888.4	2035.2	2348.5	50
T.Alkalinity (mg $CaCO_3$/l)	708.1	2662.4	1399.5	638.2	50
P.Alkalinity (mg $CaCO_3$/l)	576.5	1961.8	1038.0	421.1	50
Biogas volume (l/day)	2.7	6.8	4.7	1.2	57
CH_4 (%)	21.5	61.9	47.9	13.5	57
CO_2 (%)	38.8	67.8	49.3	9.1	57

Experimental and simulation results for pH variation and biogas production can be seen on Figures 8.2 and 8.3. Simulation values for pH show a good agreement with measurements both in stable state and during perturbations caused by the addition of sodium. For the first 27 days, the trend of the simulated pH values does not correspond to the increase observed experimentally and could be a result of the *a priori* values used for some input parameters. In day 28, the addition of $NaHCO_3$ resulted in a rapid increase of pH, and this response was correctly predicted by the modified ADM1. Subsequent pH perturbations were also accurately predicted by the model. Figure 8.3 shows a good fit between experiment and simulated values for biogas production. During the first 20 days of the study, the model tended to

underestimate the amount of biogas produced, but a better fit was obtained during periods of instability caused by the addition of sodium.

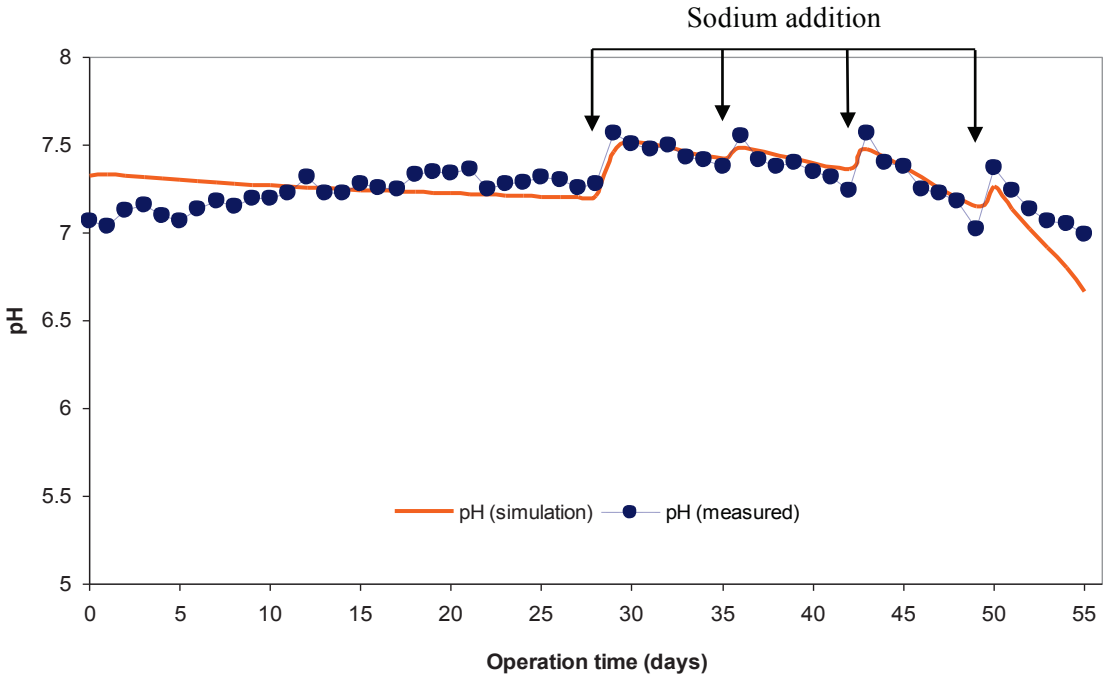

Figure 8.2. pH simulation and experimental values.

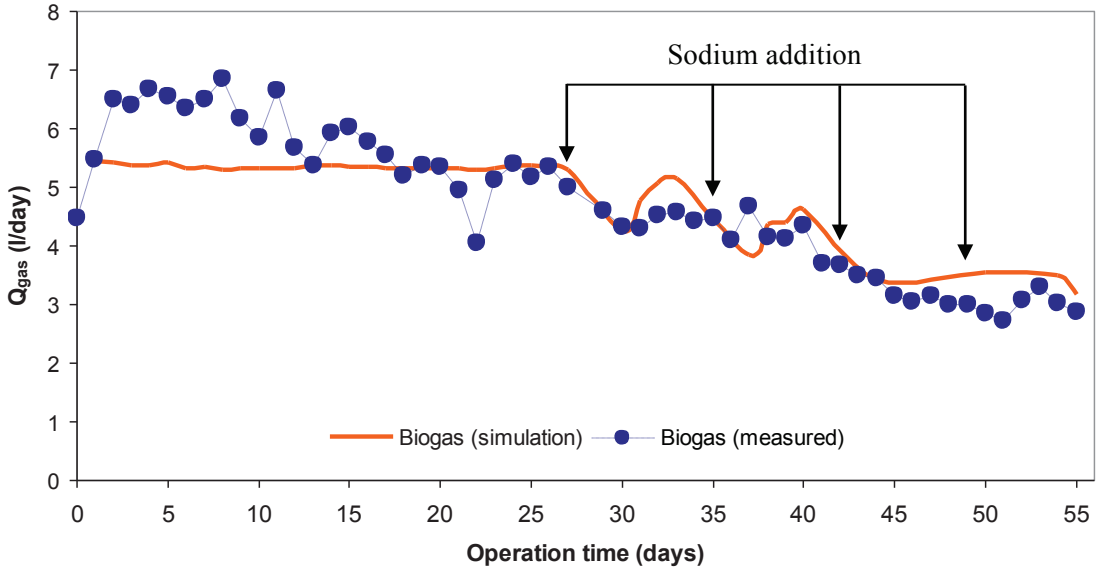

Figure 8.3. Biogas production simulation and experimental values.

Sodium addition resulted in a rapid increase of VFA as shown on Figure 8.4, an indication of inhibition on methanogenesis. Since the anaerobic inoculum used in the study was not adapted to sodium prior to the experiment, every addition of $NaHCO_3$ resulted in a rapid decrease in biogas methane content followed by a stabilisation period until the next addition as seen on Figure 8.5. Although each addition of $NaHCO_3$ might have resulted in some degree of microbial adaptation, the fact that the sodium salt was added each time as a single shock load to the system was likely to reduce the effect of adaptation on the microbial response.

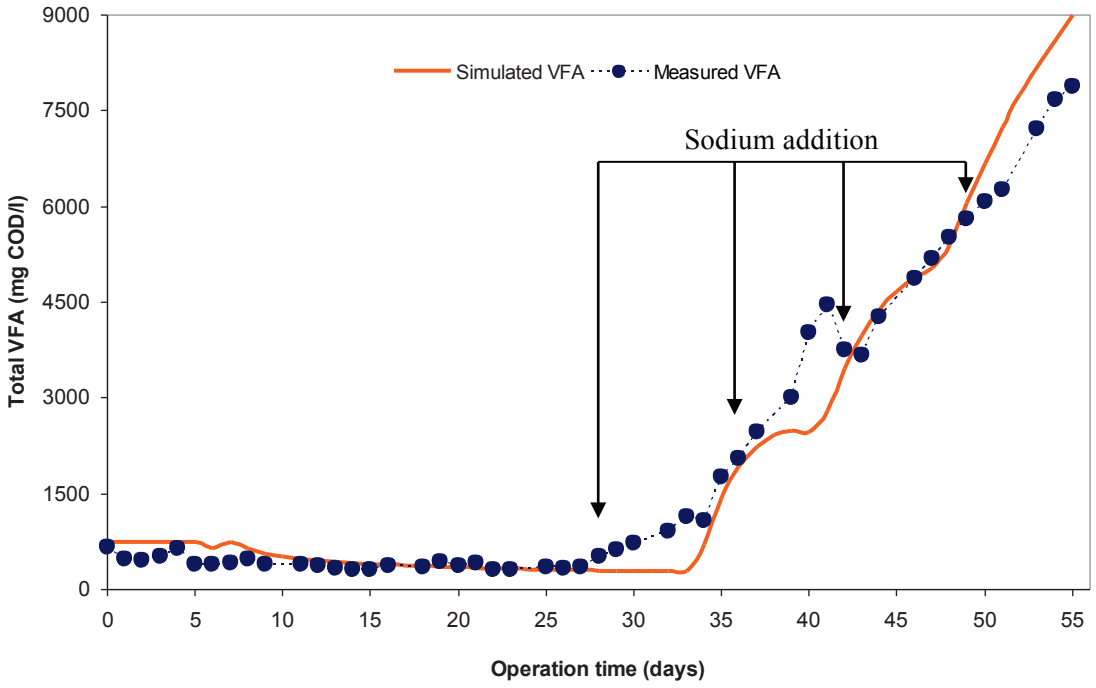

Figure 8.4. Total VFA simulation and experimental values.

During simulation, carbon dioxide was underestimated by around 10%, which could be explained by the non-optimisation of gas transfer and solubility coefficients (Blumensaat and Keller 2005). In order to reproduce the addition of sodium, it was necessary to adjust S_{CAT} since the concentration of cations was increasing

proportionally with the addition of Na^+. S_{CAT} is originally implemented in the ADM1 to represent metallic ions such as Na^+, and hence, S_{Na^+} was not directly added to the charge balance equation. Operating at high concentrations of sodium bicarbonate was interpreted by an increase in S_{IC} in the effluent with the input bicarbonate influencing the overall inorganic carbon balance.

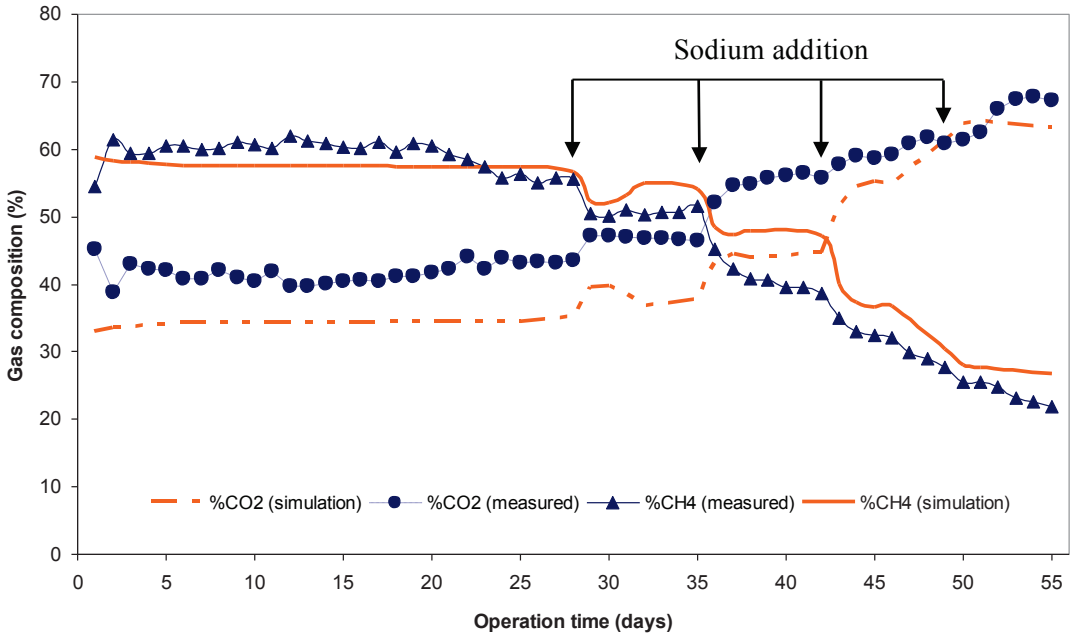

Figure 8.5. $\%CH_4$ and $\%CO_2$ simulation and experimental values.

The addition of significant amounts of $NaHCO_3$ in the system resulted in the stripping of bicarbonate in the form of CO_2, which was observed experimentally after each addition of sodium bicarbonate (Figure 8.5) and might have resulted in an increase in hydrogenotrophic activities. However, it is assumed that this pathway will be adversely affected by the increased sodium ion concentration. This seemed to be a realistic assumption, since there was a general decrease in biogas production following each addition of sodium bicarbonate, indicating that the inhibitory effect of sodium on the overall methanogenesis was greater than any increase in

hydrogenotrophic activities caused by the added bicarbonate. Table 8.3 shows a comparison of the values of some key variables from the experimental and simulation studies. The difference between experimental and simulation average values follows a normal distribution for both the biogas production and pH according to the Shapiro-Wilk test at a 5% level of significance.

Table 8.3. Reactor outputs: experimental and simulation average values.

	Parameters	Experimental	Simulation	Relative error (%)
Normal operation	pH	7.23	7.26	0
	Biogas volume (l/day)	5.73	5.34	7
	CH_4 fraction (%)	59.64	57.65	3
	CO_2 fraction(%)	41.57	34.19	18
	Total VFA (mgCOD/l)	416	441	6
Sodium addition	pH	7.23	7.16	1
	Biogas volume (l/day)	3.85	4.08	6
	CH_4 fraction (%)	39.39	43.23	10
	CO_2 fraction(%)	54.57	48.5	11
	Total VFA (mgCOD/l)	4210	4032	4

Consequently, the differences found between these values can be confidently attributed to errors in practical measurements and not to false model structure or experiment design (Koutrouli *et al.* 2009).

8.3.3. Simple and multiple linear regressions

The concentration of sodium in the reactor is found to significantly influence the biogas production at the 1% level of significance and was observed through both experimental and simulation results. Assuming that biogas production (Q_{gas}) is a dependent variable, strong negative Pearson correlations (r<-0.8) are found for Na^+ and VFA concentrations. A strong positive (r>0.7) Pearson correlation is found for methane to carbon dioxide ratio. Simple linear regressions of Q_{gas} against different

operational parameters show that significant regressions exist for all the above-mentioned parameters as can be seen on Figure 8.6.

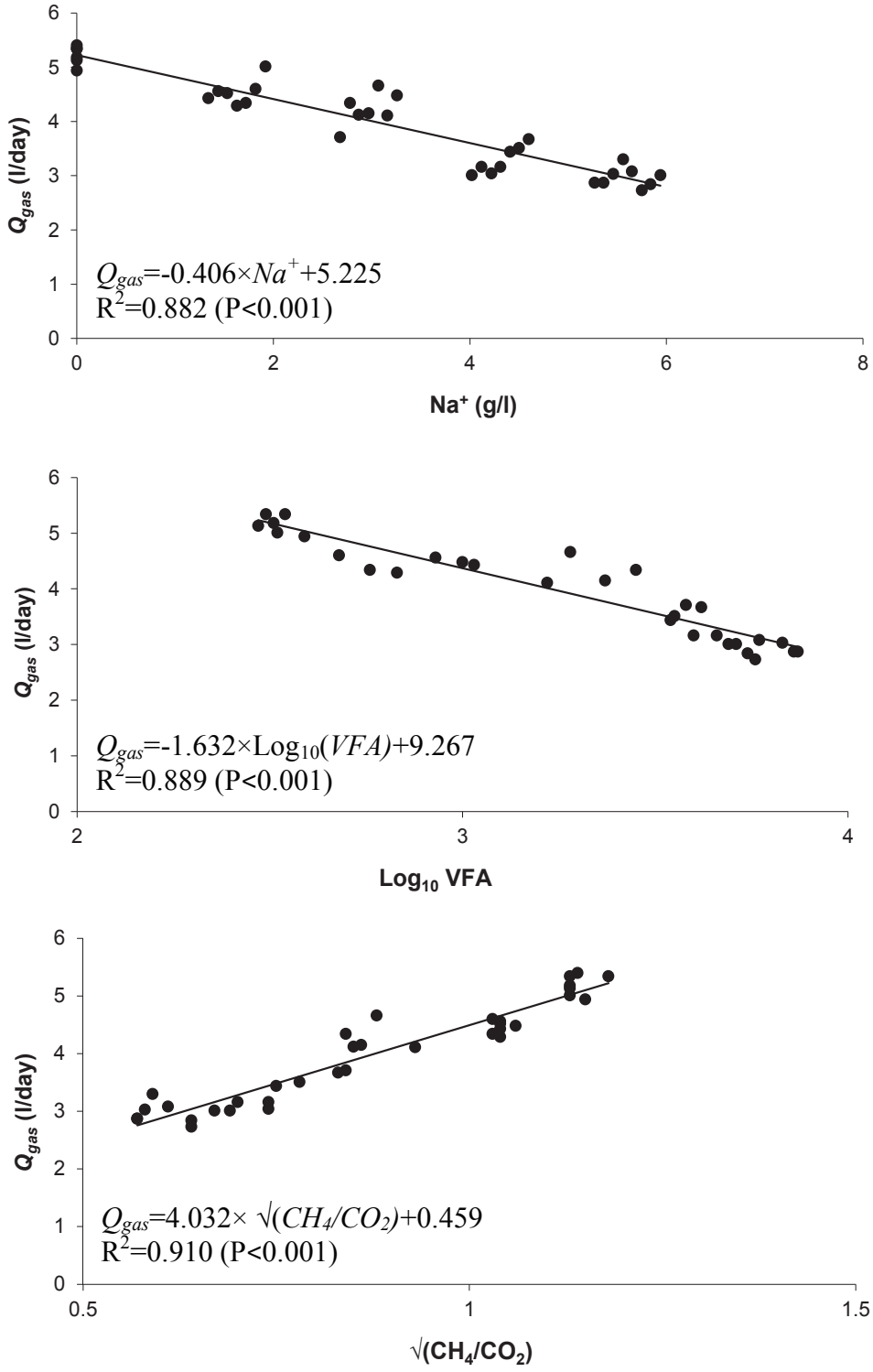

Figure 8.6. Linear regressions between biogas production and measured parameters.

Residuals for VFA concentration and CH_4 to CO_2 ratio were not following normal distribution and were therefore normalised using logarithmic and square root transformations for the latter as seen on Figure 8.6. Significant regressions were found for the CH_4 to CO_2 ratio (R^2=0.910), VFA concentration (R^2=0.889) and sodium ion concentration (R^2=0.882). This is not surprising since these parameters represent suitable indicators of the anaerobic digestion process. The methane to carbon dioxide ratio is a sensitive parameter and any sudden change in its value can indicate unbalanced conditions (Kotzé *et al.* 1969). Similarly, a fast increase in VFA concentration can be an indication of system upsetting (Cecchi *et al.* 2003). Sodium levels were also found to be strongly correlated to biogas production as a result of $NaHCO_3$ addition that impacted negatively on methanogenesis. Total alkalinity and pH were excluded from the analysis since the use of sodium bicarbonate impacted directly on both parameters and these could not be considered as indicators of process stability. However, the complexity of anaerobic degradation processes suggests that more than one parameter should be taken into account in regard to the system stability (see Chapter 2.2.3). To this extent, multiple linear regressions were conducted. A step-wise approach is often employed when using multiple regressions and the method consists of the step-by-step introduction of independent relevant parameters in the model to increase its accuracy. A combination of sodium levels, VFA concentrations and methane to carbon dioxide ratios resulted in the highest correlation found (R^2=0.938) hence, supporting the validity of this approach and the appropriateness of these parameters for process monitoring.

8.3.4. Application of the modified model in process control

Using the modified and calibrated version of the ADM1, an attempt was made to evaluate the effects of varying levels of sodium in an anaerobic digester when other known process inhibitors were present. The most common potential process inhibitor is inorganic (or ammonia) nitrogen. Ammonia is an essential macro-nutrient for microbial growth, but can also be toxic beyond certain levels. Figure 8.7 shows the average methane production over 100 days of simulation at steady state with a simultaneous variation of sodium and inorganic nitrogen concentrations and without considering the potential adaptation of the methanogenic archaea.

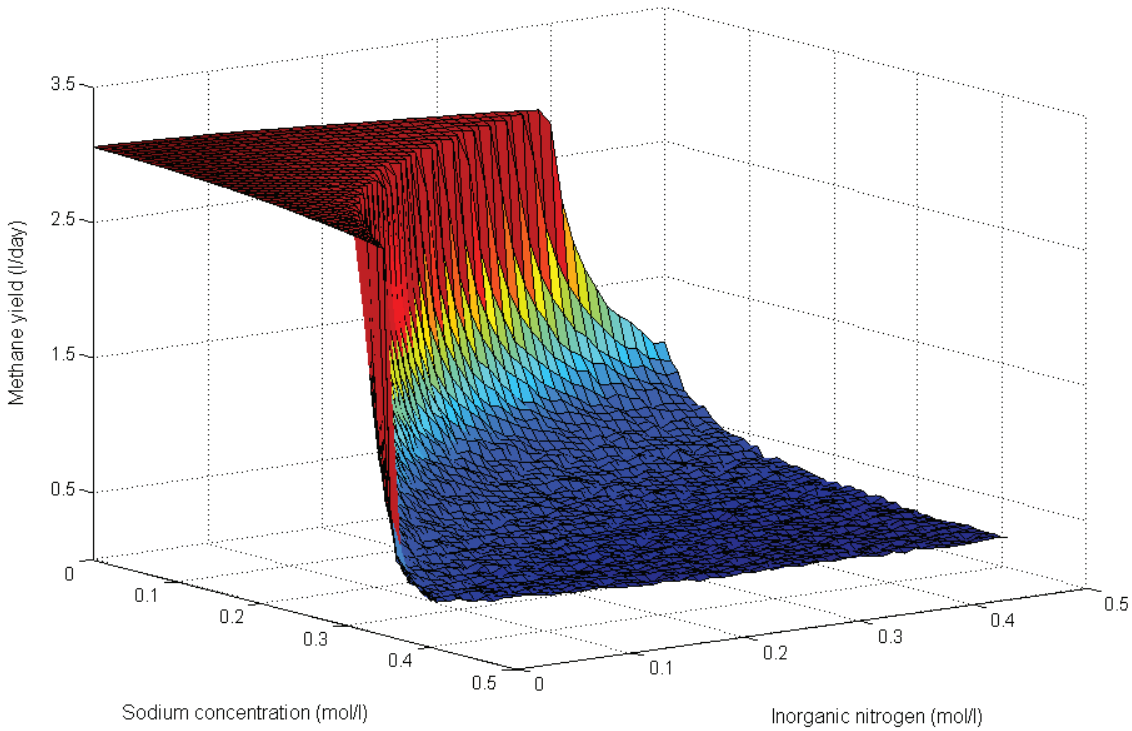

Figure 8.7. Three-dimensional representation of the effect of sodium and nitrogen concentrations on methane yield. The colour scheme used on the plot has no significance on the interpretation of the figure.

The figure shows that optimal methane yield occurs at low values of both nitrogen and sodium concentrations. A decrease of more than 90% methane produced can be noticed when operating at poor conditions. In the absence of other inhibitory compounds, substrates with low sodium ion concentrations and relatively low ammonia levels will result in higher methane yield. High methane production can also be obtained at relatively high ammonia when the sodium is low, whilst high levels of both sodium and ammonia can bring about process failure. This is particularly relevant in a context in which seaweed would be co-digested with proteinaceous substrates. Figure 8.7 also shows that the threshold inhibitory concentration for sodium ions is about 0.35 mol/l at low concentrations of inorganic nitrogen. A decrease of about 5% in methane yield is predicted at a concentration of 0.2 mol Na^+/l and is in good agreement with the decreased activity of acetoclastic methanogens observed by Rinzema *et al.* (1987) at similar sodium levels. At low concentrations of sodium, the inhibitory concentration of ammonia is found at 0.4 mol/l with literature inhibitory values ranging from 0.1 to 0.82 mol/l (Chen *et al.* 2008) and 0.25 mol/l for the most sensitive methanogens (Jarrell *et al.* 1987). However, these predicted results do not take into account possible antagonistic/synergistic effects between sodium and ammonium ions and should be considered carefully.

8.4. Conclusions

Both the experimental and modelling approaches used in this study have shown sodium ion toxicity with increasing addition of sodium salts. The ADM1 has been modified and calibrated to take into account the effect of sodium ions on acetate degrading organisms. The adjustment of a reduced set of parameters and limited experimental work has led to the accurate simulation of pH, VFA, biogas production

and composition. A good fit has been found between experimental values and simulation results at inhibitory sodium ion concentrations. Linear regression analyses further supported the observed effect of sodium ions on biogas production and the suitability of the parameters measured for process monitoring. Using the modified version of the ADM1, it has been possible to predict the effect of ammonia on sodium ion toxicity. The calibrated model predicts that relatively low sodium ion levels can bring about significant levels of process inhibition in the presence of high levels of ammonia. On the other hand, when the concentration of ammonia is relatively low, the tolerance threshold for sodium ions increases. The use of seaweed in anaerobic digestion systems might hence be negatively impacted by the inherently high concentrations of sodium ions associated with the substrate. However, co-digestion might prove an efficient practice to dilute toxic concentrations of sodium ions and the progressive adaptation of the microbial consortium to salt or the use of salt-tolerant microorganisms should also be considered.

Chapter 9
Investigating the effects of microbial adaptation to salinity

In this chapter, some of the key factors affecting the adaptation of anaerobic systems to increasing levels of salinity were identified using both experimental and modelling approaches. The ADM1 was modified to include an extra inhibition function that considers the effect of salinity and calibrated using a set of data obtained from laboratory experiments. The maximum uptake rate for acetate was adjusted to represent the lag phase in methane production observed for non-saline tolerant cultures. After calibration, the model was able to accurately predict methane production rates. The results show that, in the absence of saline-adapted inoculum, non-saline cultures can be effectively used in the start-up of anaerobic digestion systems treating moderately saline feedstocks.

9.1. Introduction

As observed in Chapter 8, the methanogenic archaea found in anaerobic reactors are particularly sensitive to sodium. Accumulation of sodium ions in anaerobic systems can bring about excessive increase in the osmotic pressure regulating the water flow across the cell membrane, which can lead to cell death (Ollivier *et al.* 1994). Some authors have reported relatively high accumulation of propionate and acetate in

anaerobic systems receiving saline feedstock, indicating that high salinity levels may have a greater impact on the acetogenic bacteria and acetoclastic methanogens than on acidogens (Rinzema *et al.* 1987; Liu and Boone 1991; Kimata-Kino *et al.* 2011). Different inhibitory concentrations of sodium ions have been reported by various authors, also suggesting that the level of microbial sodium ion inhibition may be dependent on factors such as system design, operation and seed inoculum. Soto *et al.* (1993) reported that a sodium ion concentration ranging between 14 to 18 g/l can reduce methanogenic activity by up to 50% (IC_{50}). With granular sludge, Rinzema *et al.* (1987) found an IC_{50} value of about 10 gNa^{+}/l whilst Feijoo *et al.* (1995) reported an IC_{50} of 16.3 gNa^{+}/l with saline-adapted seed inoculum. The use of an anaerobic reactor sludge bed fitted with a microfiltration membrane can increase the IC_{50} to an even higher value of 25 gNa^{+}/l (Jeison *et al.* 2008). Some microorganisms can tolerate or adapt to a relatively high sodium ion induced osmotic pressure by accumulating other inorganic ions within their cells, which ensures osmotic balance with the saline medium and thus, preventing sodium ions from reaching their cytoplasm (Oren 2002a, Oren 2002b). This may result in reduced microbial inhibitory effects of sodium ions as observed in cultures containing other cations such as Ca^{2+}, Mg^{2+} and K^{+} (Appels *et al.* 2008; Bashir and Matin 2004a; Bashir and Matin 2004b). In contrast, some microorganisms occurring naturally in highly-saline environments require sodium chloride for growth and are categorised as moderately to extremely halophilic according to the level of salt requirement (Ollivier *et al.* 1994, Kivistö and Karp 2011). Since these microorganisms, depending on their halophilic category, require certain amounts of salt for their metabolic activities, their use in wastewater treatment may only be limited to wastewaters with the requisite and less variable salt content (Aspé *et al.* 1997). Based on the characteristics of both groups of

microorganisms vis-à-vis their tolerance to saline environment, it can be assumed that halophilic anaerobic microorganisms will be more effective for treating saline rich feedstocks, whilst saline-adapted anaerobic microorganisms will adapt more readily in systems treating feedstocks containing fluctuating salt levels. The latter scenario being most often encountered in anaerobic digesters receiving diverse types of organic materials or in co-digestion systems in which the ratio of substrates might change frequently. There has been very little literature on the effect of variable levels of salts in anaerobic systems treating feedstocks which are classified as non saline-rich, such as sewage treatment plant sludge, agricultural and food/beverage residuals. However, some of these feedstocks can sometimes contain elevated levels of salts usually brought about by operational practices such as effluent recycling, pH control with sodium hydroxide and co-digesting with saline-rich feedstocks like marine biomass. The aim of this study is therefore to investigate the major factors affecting the adaptation of anaerobic systems treating non-saline rich feedstocks to variable levels of salinity using both experimental and modelling approaches.

9.2. Methodology

9.2.1. Experimental approach

The aim of the experimental approach was to determine the effects of various levels of feedstock salinity on both saline adapted and non-saline (unadapted) anaerobic cultures. The experiment was carried out in batch, using two types of inoculum: mesophilic anaerobically digested sewage sludge as non-saline inoculum (sodium ion levels of about 0.2 gNa^+/l) and digestate from a laboratory scale mesophilic anaerobic digester treating seaweed as saline-adapted inoculum (15 gNa^+/l). More information on the inoculum characteristics can be found in section 6.4. Brown seaweed

(*Laminaria digitata*) was used as a saline-rich feedstock and was prepared accordingly to the steps described in section 6.4. Different levels of feedstock salt content were obtained through dilution with either seawater or tapwater. Batch tests were designed to determine the effect of varying levels of salinity on the kinetic parameters of the anaerobic biodegradation process. Methane production rate was used as the overall indicator parameter. Ultimate methane yields were determined based on the methodology explained in section 6.1.2. Four sets of batch test cultures were used for this study. The first set consisted of culture bottles (A_{na}), each containing 400 ml of non-saline inoculum (20 g/l TS), 10 grams of the prepared seaweed, and 90 ml of tapwater. The second set of culture bottles (B_{na}), was similar to the first set, except that 90 ml of seawater, instead of tapwater, was added to each bottle. The third (A_a) and fourth (B_a) sets of culture bottles were similar to A_{na} and B_{na} respectively, except that saline-adapted inoculum (44 g/l TS) was used to replace the non-saline inoculum. All the test cultures were set in duplicate and blank test cultures containing only inoculum and water were also incubated alongside the test cultures. Methane production rate constants and ultimate methane yields were estimated using non-linear least squares curve fitting of the cumulative methane production assuming first order kinetics (see section 6.3.6). Statistical analyses were performed using the SPSS 18.0 package (SPSS International, Chicago, IL). The unpaired *t*-test was used to test the significance of differences between two samples means. All statistical analyses were preceded by the determination of model assumptions and tested at the 5% level of significance.

9.2.2. Modelling approach

The aim of the modelling approach was to predict the effects of various levels of salinity on methane production using the experimental data obtained from batch experiments. The implementation of the ADM1 in Matlab/Simulink carried out by Rosen and Jeppsson (2006) was used. Similarly to the implementation of an inhibition function considering the concentration of sodium in the model conducted in Chapter 8, an extra inhibition factor $I_{cations}$ can be applied to the rate of acetate uptake found in the original model. In addition to sodium ions, this factor takes into account the concentrations of other relevant cations such as magnesium, potassium and calcium and is expressed as shown in Equation 9.1:

$$I_{acetate} = I_{pH,ac} \cdot I_{IN,\lim} \cdot I_{NH_3} \cdot I_{cations} \qquad \textbf{(9.1)}$$

Where $I_{acetate}$ is the overall inhibition function applied to the rate of acetate uptake, $I_{pH,ac}$ is the pH-inhibition function, $I_{IN,lim}$ is the inorganic nitrogen limitation inhibition function, I_{NH3} is the ammonia nitrogen inhibition function and $I_{cations}$ is a non-competitive function taking into consideration the effect of cations concentration. $I_{cations}$ can be expressed as shown in Equation 9.2:

$$I_{cations} = \frac{1}{1 + \left(\frac{S_{Na^+}}{K_{I,Na^+}}\right) + \left(\frac{S_{Mg^{2+}}}{K_{I,Mg^{2+}}}\right) + \left(\frac{S_{Ca^{2+}}}{K_{I,Ca^{2+}}}\right) + \left(\frac{S_{K^+}}{K_{I,K^+}}\right)} \qquad \textbf{(9.2)}$$

Where S_{Na}^{+}, S_{Mg}^{2+}, S_{Ca}^{2+} and S_{K}^{+} are the concentrations of sodium, magnesium, calcium and potassium respectively found in the system. $K_{I,Na}^{+}$, $K_{I,Mg}^{2+}$, $K_{I,Ca}^{2+}$ and $K_{I,K}^{+}$ are the inhibitory concentrations of sodium, magnesium, calcium and potassium

respectively for acetate degrading organisms. Implementing an extra inhibition to the rate of acetate uptake might lead to the overestimation of the methane produced through hydrogenotrophic methanogenesis. However, it is assumed that this pathway is significantly less important than the acetic-methane pathway, since more than two thirds of methane produced during anaerobic digestion comes from the latter (Pohland 1992). Results from Chapter 8 have also shown that hydrogenotrophic methanogenesis is adversely affected by the increase of salt concentration. The function $I_{cations}$ considers that all cations present in seawater have an inhibitory effect on acetoclastic methanogens when found above their respective K_I values. In seawater cations are typically found in ratios of 0.122, 0.039 and 0.037 for Mg^{2+}/Na^{+}, K^{+}/Na^{+}, and Ca^{2+}/Na^{+} respectively (Jeison *et al.* 2008) and sodium is found at inhibitory levels before other cations reach their respective inhibitory concentrations and start to impact negatively on methanogenesis. Thus, the function is valid at cation ratios commonly found in seawater, but could be modified if the individual effects of high concentrations of magnesium, calcium or potassium need to be considered. In order to take into account possible synergism, where the presence of Ca^{2+}, Mg^{2+} and K^{+} contributes to the reduction of sodium toxicity, a different function could be implemented to decrease the value of the inhibition factor applied to acetate uptake. However, this approach would have been valid only if the synergism towards sodium ion inhibition was measured experimentally for each group of cations individually and together, and is consequently not used in this work. Sodium, calcium, magnesium and potassium cations are not added directly in the charge balance, but the parameter S_{CAT} is adjusted appropriately to obtain the correct simulation of pH within the model. This approach allows model users to obtain realistic predictions when metallic cation concentrations are not measured individually since S_{CAT} can be estimated by the

measurement of alkalinity (Hierholtzer and Akunna 2012). The concentrations of the different cations can be calculated by using Equation 8.3 individually for Na^+, Ca^{2+}, Mg^{2+} and K^+ when used in a system with a continuous flow. The degree of adaptation of the inoculum used is considered here to impact on the inhibitory sodium ion concentration tolerated by acetate degrading organisms and on their maximum specific uptake rate ($K_{m,ac}$). By adjusting the values of K_{I,Na^+} and $K_{m,ac}$ it is possible to represent the adaptation of the microbial consortium to sodium over time in the model without specific determination of microbial populations. The pathways of degradation as defined in the ADM1 can thus remain identical and do not need to take into account an alternative population of acetic acid utilising microorganisms (i.e. tolerant to sodium). This approach is appropriate for batch tests in which the sudden exposure to inhibitory substances and time scale of the study are notably different from full-scale operations.

9.3. Results and discussion

9.3.1. Experimental results

The cumulative methane production for all the cultures, net of blank results, is shown in Figure 9.1. The cultures seeded with non-saline inoculum, A_{na} and B_{na}, produced relatively small amounts of methane in the first 15 days. The delay in methane production observed for non-saline adapted cultures is believed to correspond to an acclimatisation phase during which the microorganisms were adapting to the increased salt concentration of the feedstock. After the lag period, the production of methane increased gradually and reached similar yields to those produced in cultures seeded with saline-adapted inoculum, A_a and B_a, within 50 days.

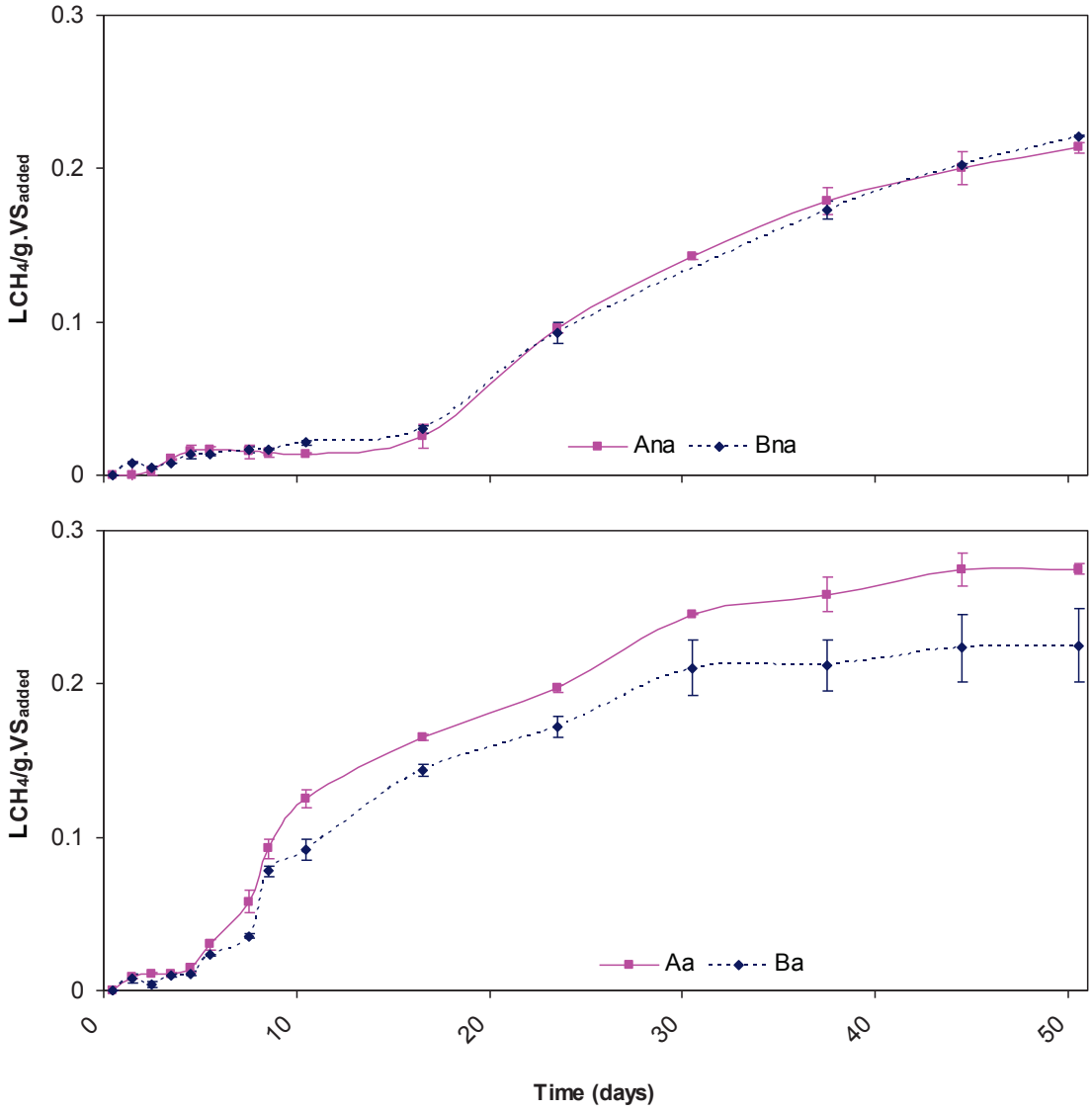

Figure 9.1. Cumulative methane production for all substrates with identical inoculum: non-saline (A_{na}, B_{na}) and saline-adapted (A_a, B_a). Error bars represent the standard deviation between duplicate experiments.

It can also be seen that, for cultures diluted with tapwater (A_{na}, A_a), a slightly greater amount of methane production was observed in cultures seeded with saline-adapted inoculum, A_a, than in those seeded with non-saline inoculum A_{na}. However, for seawater-diluted feedstock (B_{na}, B_a), there is statistically no significant difference between the methane produced by both types of inocula. Table 9.1 shows the different cations concentrations and their ratios in the cultures. It can be seen that the saline-

adapted cultures (A_a, B_a) contained significantly higher amounts of salt concentrations and dilution with tapwater or seawater had little impact on the salt levels in those cultures. The similarity in methane production obtained from B_{na} and B_a cultures (and to some extent, A_{na} and A_a) despite the significant differences in their total salt content, shows that microbial adaptation plays a major role in effective digestion of saline-rich feedstock. Hence, proper adaptation can greatly reduce the adverse effects of salinity.

Table 9.1. Cation concentrations and ratios.

Sample	Cation concentrations (g/L)				Cation ratios		
	Na^+	Ca^{2+}	Mg^{2+}	K^+	Na^+/Ca^{2+}	Na^+/Mg^{2+}	Na^+/K^+
A_{na} (tapwater)	1.22	0.08	0.24	1.23	15.25	5.08	0.99
A_a (taphwater)	13.67	0.48	0.49	2.26	28.48	27.90	6.05
B_{na} (seawater)	5.51	0.2	0.49	1.31	27.55	11.24	4.21
B_a (seawater)	14.87	0.52	0.97	2.34	28.60	15.33	6.35

Table 9.2 shows the first-order kinetics and ultimate methane yields of all cultures. For cultures seeded with saline-adapted inoculum (A_a, B_a), it was possible to assume first order kinetics and to estimate parameter values using least square curve fitting of the measured cumulative methane production. Non-saline adapted cultures (A_{na}, B_{na}) could not be considered to follow first-order kinetics from the start and parameters were calculated when biogas production started. For the saline-adapted inoculum, estimated methane yields were similar to values reported in literature for *Laminaria digitata*, which ranged between 0.26-0.29 litres CH_4/gVS_{added} (Carpentier *et al.* 1988; Chynoweth *et al.* 1993).

Table 9.2. Parameters of batch cultures.

Sample	Ultimate methane yield (Normalised l/g.VS added)	Methane production rate constant (d^{-1})	Initial pH	Final pH	VS reduction
A_{na} (tapwater)	0.212	0.034	8.5	7.5	56%
A_a (tapwater)	0.332	0.055	8.2	7.6	66%
B_{na} (seawater)	0.221	0.032	8.4	7.5	54%
B_a (seawater)	0.289	0.061	8.1	7.6	67%

Table 9.2 also shows that the levels of volatile solids reduction were comparable for similar type of inoculum (and hence, for similar salt content). The final pH values were identical for all cultures and within suitable range for methanogenesis. Table 9.3 shows the theoretical methane yields calculated from Equation 6.14 (section 6.3.5) with conversion efficiency percentages.

Table 9.3. Theoretical methane yields and calculated conversion efficiency.

Substrate (Inoculum)	Elemental composition	Theoretical CH_4 yield ($L.gVS^{-1}$)	Theoretical composition CH_4 (%)	Theoretical composition CO_2(%)	Efficiency (%)
*Laminaria digitata (*non-saline adapted: A_{na}, B_{na})	$C_{2.80}H_{5.09}O_{2.31}N_{0.18}S_{0.04}$	0.391	49.65	50.35	54.73
*Laminaria digitata (*saline adapted: A_a, B_a)	$C_{2.80}H_{5.09}O_{2.31}N_{0.18}S_{0.04}$	0.391	49.65	50.35	70.33
Literature[a,b]	$C_{2.78}H_{5.10}O_{2.36}N_{0.12}S_{0.03}$	0.397	50.00	50.00	59.70

[a]Adams *et al.* (2011a), [b]Adams et al. (2011b)

About 55% of the theoretical methane yield was obtained experimentally by using a non-saline adapted source of inoculum. This value is close to the conversion efficiency of 60% reported by Adams *et al.* (2011b). It is interesting to note that the conversion efficiency was found equal to 70% when using a salt-adapted source of seed inoculum, hence representing an increase of 15% when compared to non-adapted

inoculum. Presumably, the salt-adapted microbial consortium was able to convert most of the degradable organic matter to methane whilst non-adapted microorganisms used a greater amount of energy for survival. Vyrides and Stuckey (2009) made a similar observation when studying the role of compatible solutes and extracellular polysaccharides on anaerobic biomass and found that cells under osmotic stress consumed a significant amount of energy for adaptation and less methane was produced as a result. Thus, these results show that in the absence of saline-adapted inoculum, non-saline cultures can be successfully used for the start-up of a digester treating moderately saline feedstocks, although, in a continuously fed system, a few months adaptation period will be required before achieving optimum performance. In such a system, the adaptation period will be affected by operational parameters (such as organic loading rate or hydraulic retention time which might trigger bacterial washout) and levels of feedstock salinity.

9.3.2. Influent characterisation and parameters estimation

The ADM1 requires a careful influent characterisation together with the accurate fractionating of intermediates, namely: proteins, carbohydrates, lipids and soluble inerts. The parameters $f_{pr,xc}$, $f_{ch,xc}$, $f_{li,xc}$, $f_{si,xc}$ and $f_{xi,xc}$ were estimated from the average composition of *Laminaria digitata* used in the study. First order parameters corresponding to the hydrolysis kinetic rates of carbohydrates, lipids and proteins were set at similar rates since their influence is not significant for homogenous substrates (Blumensaat and Keller 2005; Feng *et al.* 2006). $K_{hyd,ch}$, $K_{hyd,pr}$, $K_{hyd,li}$ were calibrated from the cumulative production of methane obtained after 50 days of experiment, as shown in Figure 9.1. The feedstock characterisation was determined by using the transformer model elaborated by Zaher *et al.* (2009a). The input

characterisation resulting from experimental measurements and the yield of products on substrates are given in Table 9.4. The concentrations of sodium, potassium, magnesium, and calcium were adjusted from measured values shown in Table 9.1.

Table 9.4. Input characterisation and stoichiometric parameters.

Parameter	Value	Definition
Input characterisation[a]		
$S_{su,in}$	20.33 kgCOD.m^{-3}	Sugar concentration
$S_{ac,in}$	0.63 kgCOD.m^{-3}	Acetate concentration
$S_{ic,in}$	0.02 M	Inorganic carbon concentration
$S_{in,in}$	0.28 M	Inorganic nitrogen concentration
$S_{I,in}$	15.93 kgCOD.m^{-3}	Soluble inert concentration
$X_{I,in}$	7.03 kgCOD.m^{-3}	Particulate inert concentration
$S_{cat,in}$	0.08 M	Cations concentration (excluding cations added from seawater)
$S_{an,in}$	0.003 M	Anions concentration
Yield of product on substrate		
$f_{si,xc}$	0 kgCOD.kgCOD^{-1}	Yield of soluble inerts on composites
$f_{xi,xc}$	0.35 kgCOD.kgCOD^{-1}	Yield of particulate inerts on composites
$f_{ch,xc}$	0.42 kgCOD.kgCOD^{-1}	Yield of carbohydrates on composites
$f_{pr,xc}$	0.21 kgCOD.kgCOD^{-1}	Yield of proteins on composites
$f_{li,xc}$	0.02 kgCOD.kgCOD^{-1}	Yield of lipids on composites

[a] All other state variables are taken equal to 0

The maximum uptake rate for acetate ($K_{m,ac}$) was adjusted by fitting simulation results to experimental results, particularly VFA and methane production. The values of $K_{m,ac}$ obtained were 17±0.82 kgCOD.kgCOD^{-1}d^{-1} and 7.83±1.55 kgCOD.kgCOD^{-1}d^{-1} for saline-adapted and non-saline cultures respectively. The much lower value obtained for the non-saline inoculum is due to the fact that the acetic acid utilisers not adapted to salinity showed a delay (or adaptation period) before methane production started. A similar strategy was used by Batstone *et al.* (2003) to represent an observed delay in the uptake of valerate by adjusting both half saturation value (K_s) and uptake rate.

Increasing the half saturation value of acetate utilisers was not considered since it has been reported that different pairs of K_m / K_s could yield similar simulation results with both parameters being mathematically dependent and impossible to calibrate simultaneously (Girault *et al.* 2011). Hence, the values of K_m used in this study were valid only when K_s was set at its default value (0.02 day^{-1}). Similarly, the 50% inhibitory concentration for sodium was estimated from experimental results and adjusted to 0.11±0.009 $kmol/m^3$ for non-saline microorganisms and 0.61±0.005 $kmol/m^3$ for the salt-adapted archaea. Inhibition parameters for other cations were taken from literature with $K_{I,Mg}{}^{2+}$=0.06 $kmol/m^3$ (Appels *et al.* 2008), $K_{I,Ca}{}^{2+}$=0.12 $kmol/m^3$ (Ahn *et al.* 2006) and $K_{I,K}{}^{+}$=0.15 $kmol/m^3$ (Kugelman and McCarty 1965). Initial values for inorganic carbon (S_{IC}), inorganic nitrogen (S_{IN}), and cation concentrations (S_{CAT}) were obtained from experimental values. All other parameters were taken equal to the values used by Rosen *et al.* (2006) or suggested by Batstone *et al.* (2002).

9.3.3. Simulation results

Figure 9.2 shows experimental and simulated values of the cumulative methane produced in non-saline adapted cultures using both the original and modified version of the ADM1 model. A good fit was obtained between the results of the modified model and the experimental data. It can also be seen that the model correctly predicted the lag phase that was observed in the experimental results.

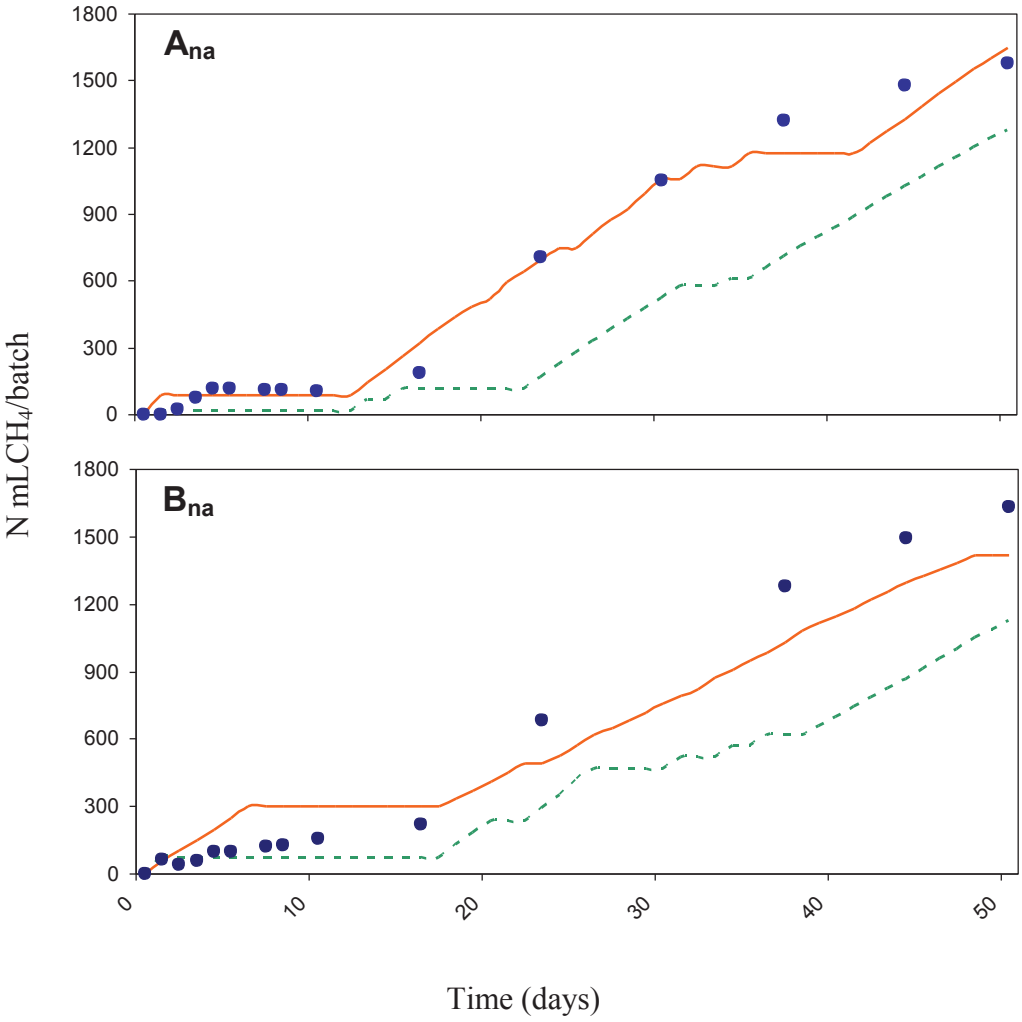

Figure 9.2. Cumulative methane production from experimental (•), modified ADM1 (—) and original ADM1 (----) data for the non-acclimatised inoculum cultures diluted with tapwater (A_{na}) and seawater (B_{na}).

Figure 9.3 shows the model and experimental results for the saline-adapted cultures. The first 10 days of experiment were predicted correctly by the model. However, methane production was underestimated between days 10 and 40. The original model systematically predicted much lower production of methane.

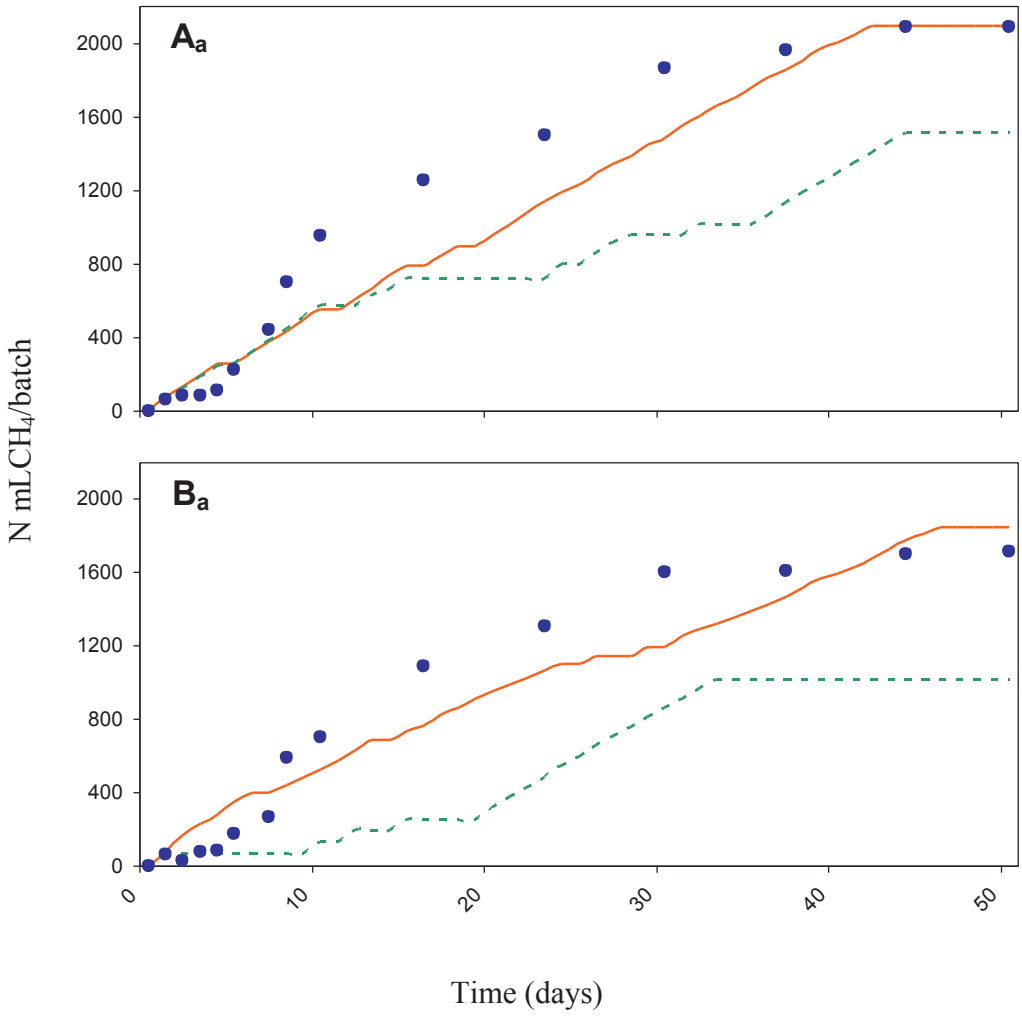

Figure 9.3. Cumulative methane production from experimental (•), modified ADM1 (—) and original ADM1 (----) data for the salt-tolerant inoculum cultures diluted with tapwater (A_a) and seawater (B_a).

Figure 9.4 shows the correlation between methane productions predicted by the modified ADM1 and measured values. The high correlation coefficients ranging from 0.925 to 0.985 is indicative of the validity of the modelling approach and parameters estimation method used in this study.

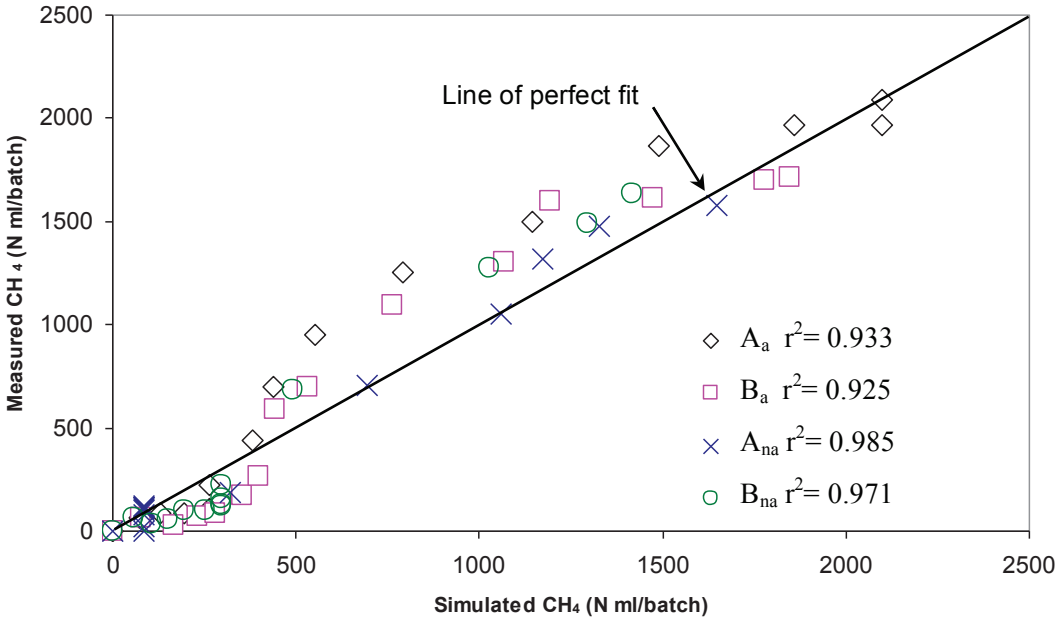

Figure 9.4. Comparison between experimental and modified ADM1 model results for methane production.

9.4. Conclusions

Both the experimental and modelling approaches used in this study have shown that non-saline adapted anaerobic sludge can be effectively used in the start-up of anaerobic digestion systems treating saline enriched feedstocks, following a minimum period of adaptation. After modification and calibration of the ADM1, a good fit has been found between experimental and modelling values as evidenced by high correlation coefficients. The modified model has also been able to correctly predict the lag phase period associated with microbial adaptation to salinity (i.e. for non saline inoculum) during which little or no methane gas is produced. Seaweed can therefore represent an effective feedstock for biogas production provided a sufficient period of adaptation is allowed.

Chapter 10
Impact of phenolic compounds on anaerobic digestion

This chapter addresses the effect of phenolic compounds on the anaerobic digestion process. The specific effects of two types of phenolic compounds on mixed microbial cultures found in anaerobic systems are investigated. Assays were conducted with phloroglucinol, as the non-polymerised form of phlorotannin, and with phlorotannins extracted from the brown seaweed Laminaria digitata. Electron micrographs obtained from the study revealed that phlorotannins induce significant extra- and intra-cellular effects upon cells, with the disruption of cell membranes observed with most microorganisms. Results also suggest that phloroglucinol strongly affects cell membrane permeability. By monitoring intermediary compounds during the anaerobic digestion of phlorotannins, it was also found that higher energy consumption is required by microorganisms for survival under stress induced by these phenolic compounds.

10.1. Introduction

Polyphenolic compounds are known inhibitors of anaerobic digestion systems (Chen *et al.* 2008). Their detrimental effects on anaerobic microorganisms have been observed in anaerobic systems treating coal gasification wastewater (Wang and Han

2012), olive mill wastes (Fezzani and Ben Cheikh 2007) or wine distillery wastewater (Borja *et al.* 1993). Higher molecular weight polyphenols of natural occurrence have also been found to be toxic towards methanogens (Field and Lettinga 1987). Phloroglucinol derived chemicals are polyphenolic compounds and are used in medicine, cosmetics, paints, pesticides and as dyestuffs. The group includes about 700 naturally occurring variations (Singh *et al.* 2010). The phloroglucinol parent molecule (1,3,5-trihydroxybenzene) has been isolated from numerous natural sources (Singh and Bharate 2006) with the most complex polymerised forms, phlorotannins, exclusively found in brown seaweed species (see section 3.3.3). Phlorotannins, as most phenolic compounds, are likely to affect cell membrane activity inducing leakage of intracellular constituents (McDonnell and Russell 1999; McDonnell 2007). The cytoplasmic membrane is highly active metabolically and acts as a selective permeability barrier with the cell's external environment, that once damaged, results in the loss of intracellular potassium, 260 nm absorbing materials (mainly nucleic acids) and phosphates (Rye and Wiseman 1964; Elferink and Booij 1974; Al-Adham *et al.* 1998). Wang *et al.* (2009) observed damaged cell walls of *Escherichia coli* cells exposed to phlorotannins extracted from the brown seaweed *Ascophyllum nodosum*. However, the mode of action of phlorotannins on anaerobic microorganisms remains obscure and there is little information available regarding their influence on mixed microbial cultures found in anaerobic digesters. In this study, the inhibitory effects and possible mode of action of brown seaweed phlorotannins on mixed anaerobic microbial cultures are investigated. The relationship between antibacterial activity and the degree of polymerisation of phlorotannins is also studied. Factors investigated include the production of methane at various polymerisation levels and concentrations of phlorotannins extracted from the seaweed *Laminaria digitata* and cell membrane

damage through the monitoring of 260 nm absorbing materials and transmission electron microscopy observations. Interactions between proteins and phlorotannins are also studied using absorbance profiles at ultraviolet wavelengths.

10.2. Methodology

10.2.1. Batch studies

The experimental batch tests were designed to monitor the biodegradability and possible impact of phlorotannins on acetoclastic methanogens through the measurement of cumulative methane production. Phlorotannins were extracted according to the method described in section 6.5. In order to investigate the effect of the degree of polymerisation on antibacterial activity, tests were also conducted with anhydrous phloroglucinol (Sigma, UK) as a control for the non-polymerised form of phlorotannins. The assay was conducted according to the method described in section 6.1.2 with some modifications. The inoculum (17 g/l TS), as detailed in section 6.4, was left to starve for 24 hours in basal medium. Thereafter, different concentrations of phloroglucinol (50 to 1000 mg/l) or phlorotannins (20 to 200 mg/l) were added to the bottles together with 25 ml of sodium acetate solution (concentration 2g COD/l). Concentrations of phloroglucinol were selected in accordance to previous observations in which phloroglucinol was rapidly acidified and later converted to methane at concentrations as high as 1500 mg/l (Field and Lettinga 1989). The addition of a model substrate, such as acetate, was used to assess the activity of the trophic group of interest, i.e. acetoclastic methanogens. Specific biogas potential of phloroglucinol and phlorotannin were not assessed. A non-growth synthetic medium adapted from Akunna *et al.* (1993) was used to provide essential nutrients, as listed in section 8.2.1. The batch cultures were set in duplicate and incubated at mesophilic

temperatures for 30 days along with controls containing no phloroglucinol or phlorotannin.

10.2.2. Protein binding assay

The binding of phlorotannins to proteins was assessed by observing the emission spectra of solutions containing a known concentration of albumin from bovine serum (BSA) (Sigma, UK) and different levels of phlorotannins. BSA in a lyophilised form was diluted in deionised water to obtain a concentration of 3 mg/ml. One millilitre of the solution was then added to 2 ml of a solution containing either deionised water (control) or phlorotannin (ligand) at different concentrations (0.1-50 mg/l) to give a known final reaction concentration in 3 ml. The solution was transferred in a clean quartz UV cuvette to measure absorbance between 220 to 320 nm in a calibrated spectrophotometer.

10.2.3. Cell membrane leakage assay and electron microscopy

Cell membrane leakage assays consisted of the measurement of potassium (K^+) fluxes and the leakage of 260 nm absorbing material over time. The method is fully described in section 6.1.3. Transmission electron microscopy was used to observe the effect of phlorotannins on mixed anaerobic microbial cultures and cell suspension preparation for microscopy analysis is detailed in section 6.1.4. Mixed anaerobic microbial cultures were obtained from the same source of inoculum used for the batch assays and exposed to phloroglucinol (final concentration 1000 mg/l), phlorotannins (final concentration 18 mg/l) or water (control). Statistical analyses were performed using the SPSS 18.0 package (SPSS International, Chicago, IL), the unpaired *t*-test was used to test the significance of differences between two samples means. The one-

sample *t*-test was used to assess the significance of cell leakage results. All statistical analyses were preceded by the determination of model assumptions and tested at the 5% level of significance.

10.3. Results and discussion

10.3.1. Methane production and intermediary indicators

The cumulative methane production at different levels of phloroglucinol and phlorotannins can be seen in Figure 10.1. Results are expressed as litres of methane measured per gram of chemical oxygen demand (COD) added at standard temperature and pressure for comparison. The test was stopped after 30 days when a significant production of methane could no longer be observed. In the presence of phloroglucinol or phlorotannins, there is no statistically significant difference of methane production between the controls and tested levels. However, ultimate methane volumes found at the highest concentration of phlorotannins were 20% lower when compared to control levels, whilst a maximum of 10% variation in final methane yields could be found at phloroglucinol concentrations tested and when compared to control. At a concentration of 1000 mg/l phloroglucinol, it can be seen that methane production rates were lower for the first few days of the assay. This observation is confirmed by the value of the first order degradation constant *k* which was found to be equal to 0.27 day^{-1} for the highest phloroglucinol concentration and 0.39 day^{-1} for the control. For phlorotannins, *k* was in the range of 0.32-0.41 day^{-1}.

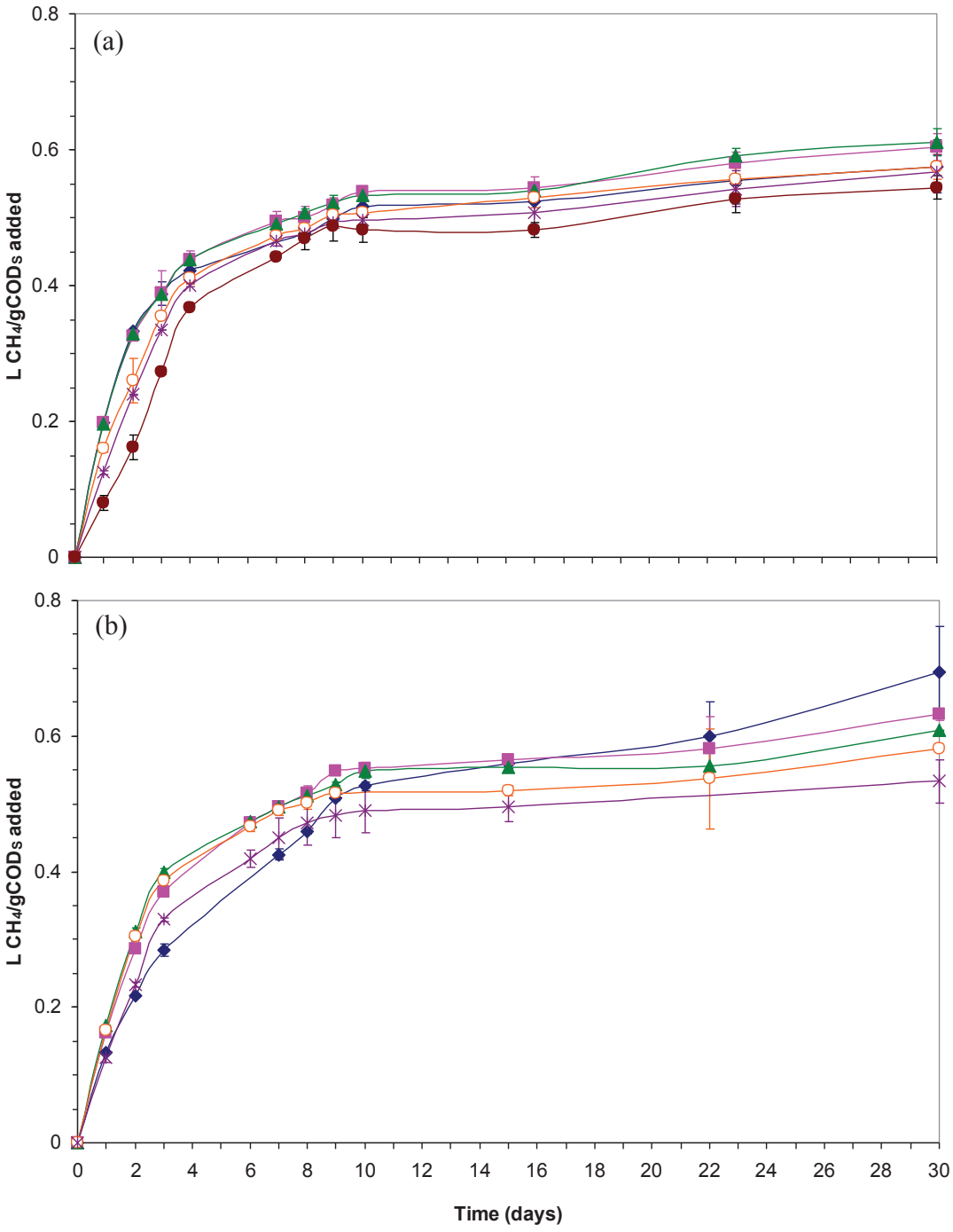

Figure 10.1. Cumulative methane production with different phloroglucinol concentrations (a): ◆ control; ■ 50 mg/l; ▲ 100 mg/l; ○ 250 mg/l; × 500 mg/l ; ● 1000 mg/l and phlorotannins concentrations (b):◆ control; ■ 20 mg/l; ▲ 50 mg/l; ○ 100 mg/l; × 200 mg/l. Error bars represent the standard deviation between duplicate experiments.

Changes in volatile fatty acids (VFA) levels can be used to monitor the progress of anaerobic degradation and Figure 10.2 shows the variations of total VFA during the assay.

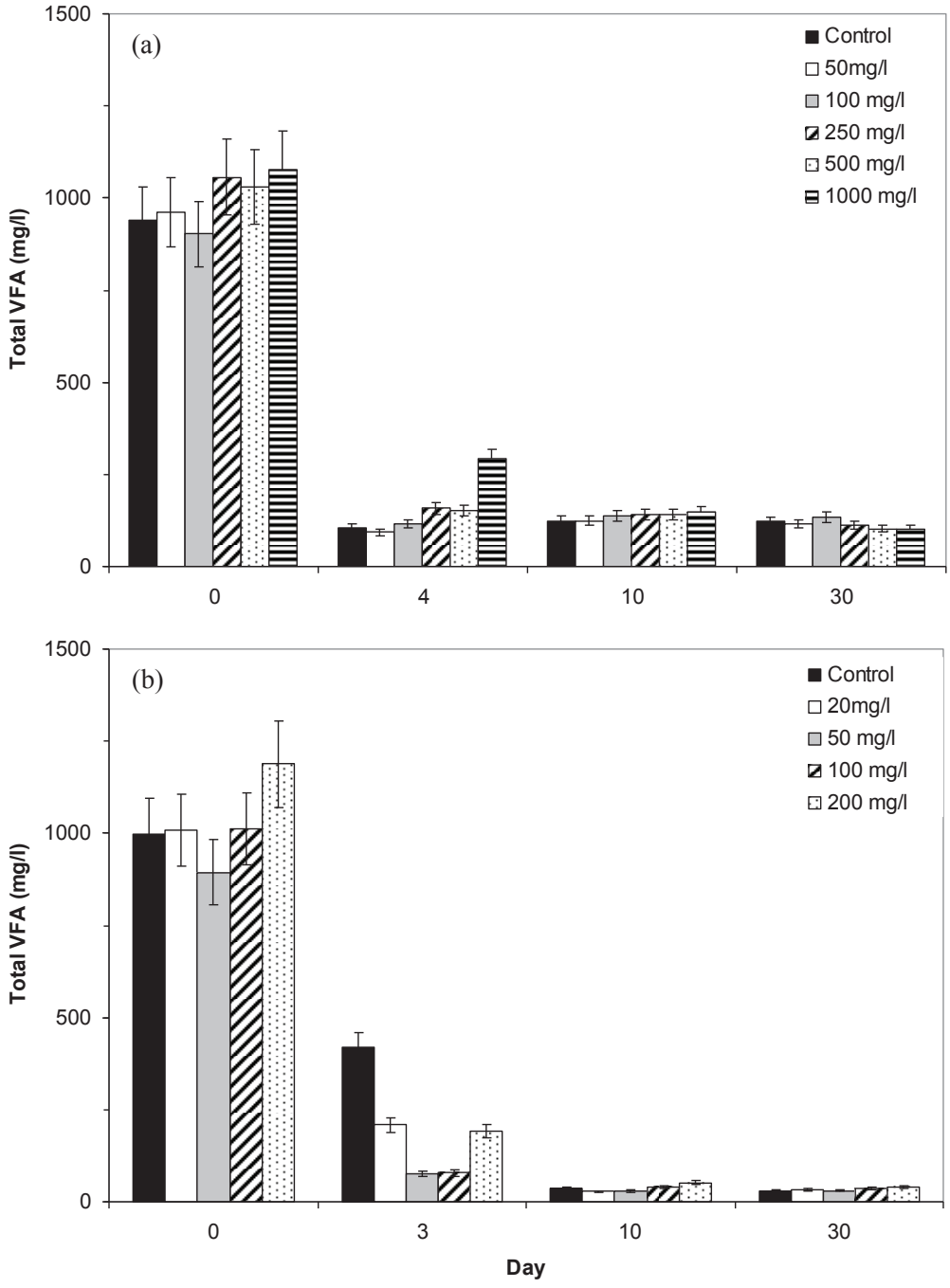

Figure 10.2. VFA profiles at different concentrations of phloroglucinol (a) and phlorotannins (b). Error bars represent the standard error associated with the method of measurement.

It can be seen that VFA levels at the beginning of the experiment are similar for all groups and about 1000 mg/l acetic acid (HAc). For cultures exposed to phlorotannins, VFA profiles show no accumulation and levels rapidly decreased for all groups, presumably due to the rapid conversion of acids to methane. At the highest concentration of phloroglucinol, the decrease of VFA levels was slower than observed for the control, but concentrations were similar for all groups from day 10. This observation is consistent with the delay observed for methane production at 1000 mg/l phloroglucinol during the first days of the assay. The pathway of phloroglucinol degradation under anaerobic conditions is believed to consist in its conversion to a phenol intermediate by removal of ring substituents, followed by ring fission and formation of cyclohexanol and cyclohexanone, yielding organic acid metabolites which are later converted to methane via methanogenesis (Young and Rivera 1985) as shown by Figure 10.3.

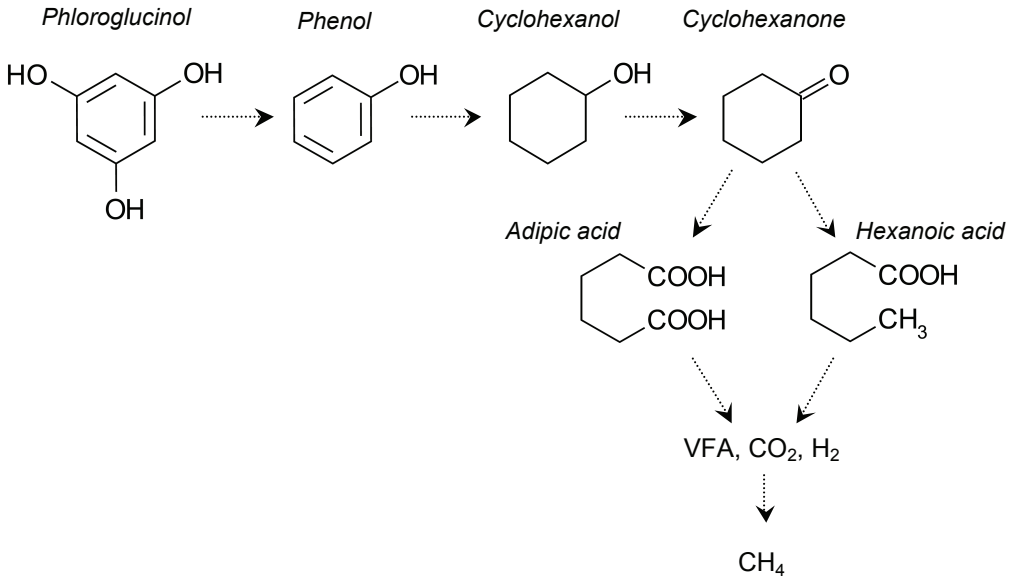

Figure 10.3. The metabolic fate of phloroglucinol under anaerobic conditions (adapted from Young and Rivera 1985).

It is reasonable to assume that any inhibitory effect induced by phloroglucinol will tend to decrease once the benzene ring has been cleaved and the original compound is metabolised to methane precursors by tolerant microorganisms. This would explain the kinetic inhibition observed for methane generation and VFA removal during the first eight days of experiment and the similar levels of cumulative methane at the end of the experiment. The degradation of phlorotannins presumably follows a similar pathway, but the metabolism of high-molecular weight phenolic compounds is likely to depend on polymerisation levels with ring fission being more complex for highly polymerised compounds. However, similar initial biogas production and VFA removal show that no inhibitory effect could be observed during the first few days of the test, whilst the amount of methane produced at the end of the experiment was inversely proportional to phlorotannin levels. During anaerobic digestion, biomass synthesis typically consumes 5% of the organic matter (Symons and Buswell 1933) and most of the remaining degradable fraction is expected to be transformed into methane if favourable conditions for methanogenesis are provided. An indication of organic matter levels is obtained through the monitoring of chemical oxygen demand and a stoichiometric conversion to methane is usually assumed. In this experiment, final levels of COD_S were found to be similar (350 mg/l) in all phlorotannin treated cultures, indicating that most of the organic matter was used for methane generation or biomass growth with only a small non-biodegradable fraction remaining. This suggests that whilst most of acetic acid was removed and COD_S consumed, a smaller fraction of organic matter could be converted to methane in cultures containing phlorotannin. When assessing the impact of antimicrobials on acetoclastic methanogens Cetecioglu *et al.* (2012) observed the removal of acetic acid, even when almost no methane was produced. The authors interpreted the phenomena to be an

uncompetitive inhibition caused by the antimicrobials tested. It is known that under unfavourable conditions, cells can synthesise organic solutes for survival, but energy requirements are consequently increased and metabolic activity is likely to be reduced (Muller *et al.* 2005). In the light of the COD levels found during this experiment, this latter theory is the most probable, as the methane production and VFA removal results indicate that higher energy consumption was required by microorganisms for survival under stress induced by phlorotannins. Field *et al.* (1989) found that small oligomers were more toxic at early stages than more highly polymerised compounds closer to high molecular weight tannins. A similar observation can be made in this study with a kinetic inhibition triggered by phloroglucinol at the beginning of the assay opposed to the residual impact of phlorotannins.

10.3.2. Cell membrane leakage

Figure 10.4 represents the absorbance at 260 nm for solutions containing mixed anaerobic cultures with different concentrations of phlorotannins and phloroglucinol. The variations of absorbance were statistically tested against their initial value at the beginning of the assay. In solutions with different concentrations of phlorotannins, a significant variation in absorbance could only be observed from the solution containing 70 mg/l phlorotannins, where a peak in absorbance was found after 5 minutes of exposure. Subsequent absorbance decreases could be a result of the coagulation of protoplasmic contents resulting in smaller leakage rates (Hugo and Longworth 1965). No significant increase in 260 nm absorbance could be found at other phlorotannin concentrations. However, it has been observed that after the addition of phlorotannin, the cell suspension immediately started to coagulate.

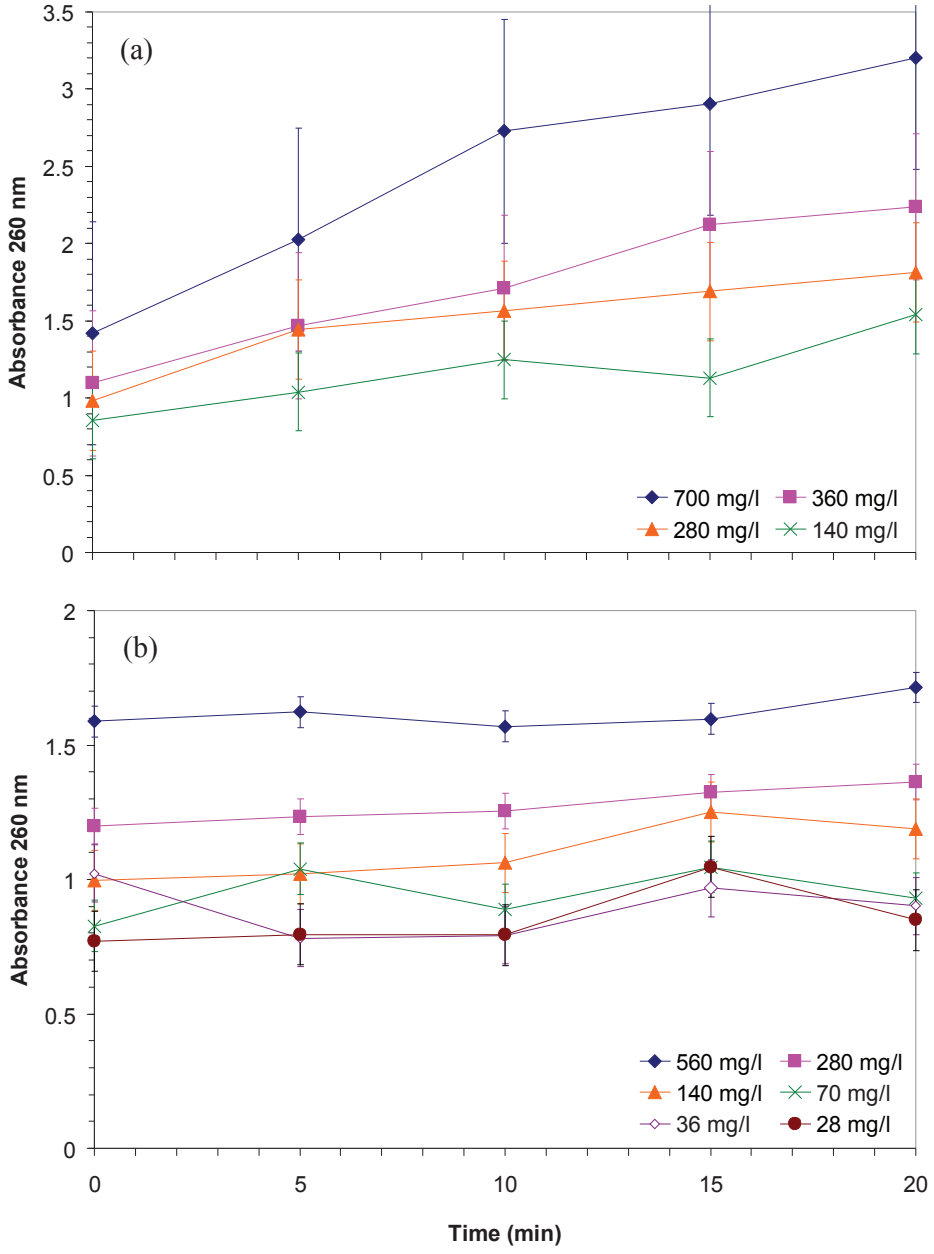

Figure 10.4. Leakage of 260-nm-absorbing material from solutions of mixed anaerobic microorganisms exposed to different levels of phloroglucinol (a) and phlorotannins (b). Error bars represent the standard error of the data set.

Due to the experimental procedure used, the pellet formed was removed by centrifugation before measurement of the supernatant, which could have resulted in the removal of leakage material by sedimentation. Significant increases in absorbance were found at concentrations equal to or higher than 280 mg/l phloroglucinol, which suggests a cell membrane permeability effect. The absorbance at the beginning of the

assay is different for all concentrations tested, since both phlorotannin and phloroglucinol have been found to have absorbance peaks around 260 nm, thus impacting on the original absorbance monitored during leakage assays. Figure 10.5 shows the absorbance of phloroglucinol and samples of phlorotannins extracted from *Ascophyllum nodosum* and *Laminaria digitata* collected from the same location at a year interval. The compounds were analysed at a wavelength spectrum comprised between 240 to 380 nm.

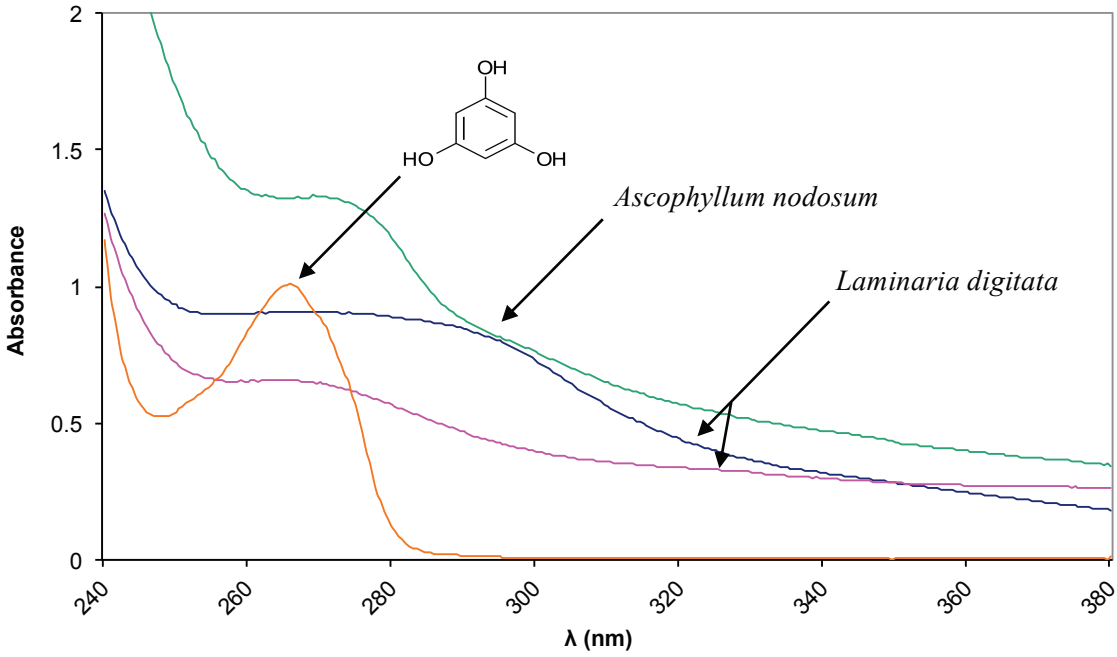

Figure 10.5. Absorbance profiles of phloroglucinol (—), phlorotannins extracted from *Ascophyllum nodosum* (—) and phlorotannins extracted from *Laminaria digitata* collected from the same location at a year interval. (— / —).

Phlorotannin related compounds absorbed light at varying wavelengths, but all had their absorption maxima between 270 and 280 nm, whereas phloroglucinol had its absorption maxima at 266 nm. These results are similar to the observations made by Koivikko (2008), who found identical absorption profiles for phlorotannins extracted from *Fucus vesiculosus*, and reported the maximum absorbance of phloroglucinol at

270 nm. However, the identification of phlorotannin compounds is not possible from their UV spectra alone, since polymeric standards for phlorotannins are not commercially available.

Potassium ion effluxes were also monitored at different concentrations of phloroglucinol and phlorotannins, but K^+ levels remained relatively constant during the experiment (hence, results not shown). This observation could be explained by the fact that phloroglucinol and its polymeric derivatives might act as ion chelating agents. Ragan *et al.* (1979) observed that high-molecular polyphenols extracted from the brown seaweed *Ascophyllum nodosum* can chelate a wide range of divalent cations and ion chelating was also reported by others (Senevirathne *et al.* 2006; Chew *et al.* 2008). Hence, the leakage of potassium ions would not be noticeable because of their immediate uptake or chelation by phloroglucinol or phlorotannins. In the light of 260 nm absorbing material results found for phloroglucinol (Figure 10.4.a), the latter assumption seems reasonable.

Interactions between phlorotannins and proteins were also investigated, but experimental results (not shown) proved inconclusive. The methodology used relied on the measurement of the absorbance of solutions containing BSA and varying levels of phlorotannins. However, the absorbance peaks of both compounds are similar and around 275 nm (Figure 10.5). Thus, the addition of phlorotannins at increasing levels impaired the spectrophotometric readings and no significant changes in absorbance or new emission peaks resulting from the binding of phlorotannins to proteins could be observed. Moreover, binding studies generally involve the calculation of association/dissociation constants and the determination of the number of phenol molecules that bind to the different binding sites of the protein molecule (Rawel *et al.* 2005). This could only be achieved by knowing the exact molecular weight of the

ligand (i.e. phlorotannins), which was not determined in this study. The ability of some phlorotannins to oxidise and form covalent bonds with proteins has been observed by Stern *et al.* (1996b).

10.3.3. Microscopy observations

Comparison of control cells in Figure 10.6 and cells exposed to phloroglucinol in Figure 10.7 suggests that microorganisms were affected by phloroglucinol and supports the observation of membrane leakage in earlier results. Field shots of bacterial cells from Figure 10.6a (control) show the diversity of microorganisms found in the mixed anaerobic cultures, with normal cellular morphology observed at higher magnification (Figure 10.6c) and a smooth continuous cell envelope structure (Figure 10.6d). Figure 10.7a (phloroglucinol exposed cells) shows many spore-like structures and cells with disrupted outer membranes. These observations are further elucidated at higher magnification in Figure 10.7b, with evident signs of membrane structure disruption (A) and the coagulation of exopolysaccharides (B). Cells exposed to phloroglucinol exhibit signs of membrane dysfunction as seen in Figure 10.7c with separation of the cytoplasmic membrane from the cell envelope (A) and membrane 'blebbing' (B). Figure 10.7d shows fine details of an endospore created by microorganisms under unfavourable nutritional or environmental conditions. These observations suggest that phloroglucinol interacts with bacterial envelope and triggers survival mechanisms such as sporulation for affected cells.

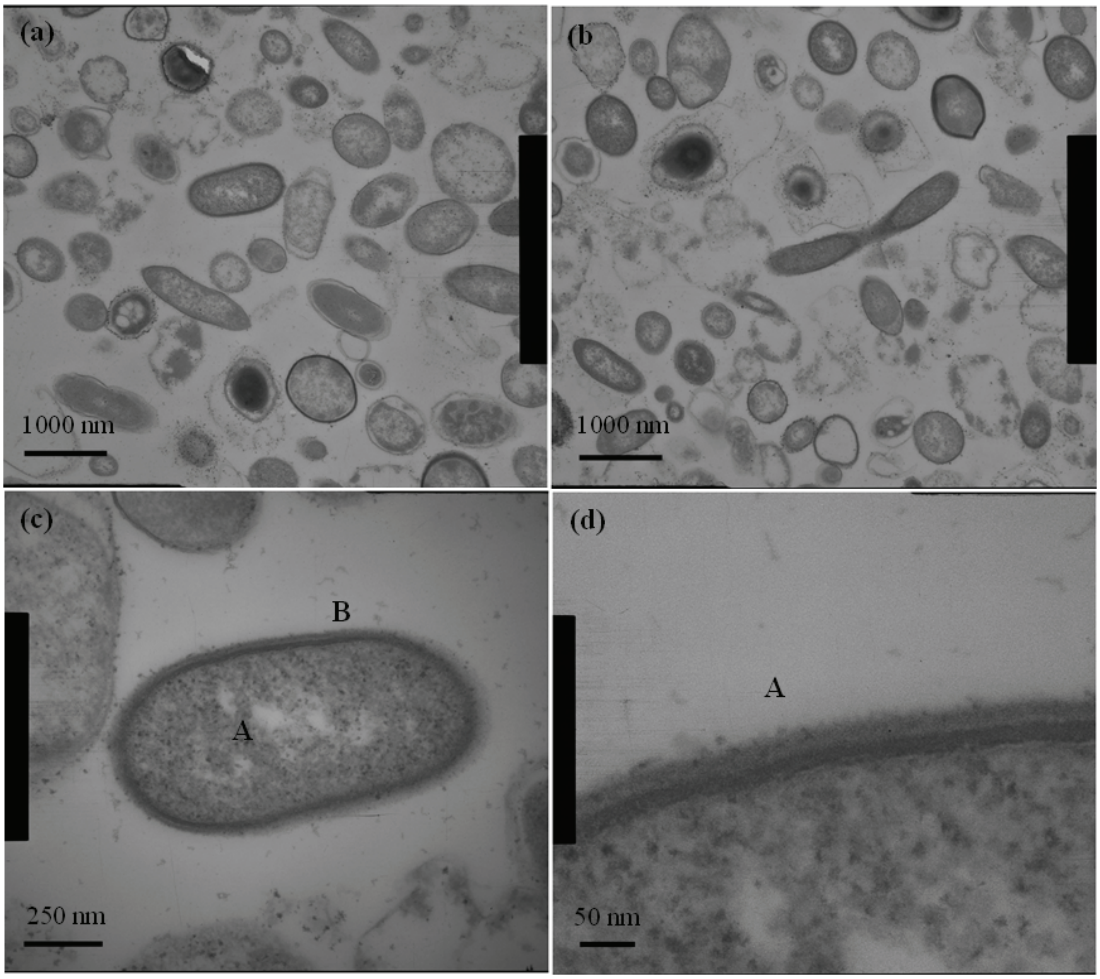

Figure 10.6. Transmission electron micrographs of mixed anaerobic microbial cultures exposed to water at 15000× magnification (a & b), 60000× magnification (c) and 200000× magnification (d). Figure 10.6(c) shows normal cytosolic components (A) and an intact cell envelope (B). Figure 10.6(d) shows fine details of the intact envelope structure (A).

However, cell membrane interactions are influenced by the bacterial cell walls structure, i.e. Gram-negative or Gram-positive, and cells seem to be affected to a different extent depending on their general morphology. In the light of the cumulative methane production obtained during batch tests (Figure 10.1a), this would confirm that the toxicity of phloroglucinol decreases through its degradation by tolerant microorganisms inducing benzene ring fission and later conversion to methane (Figure 10.3).

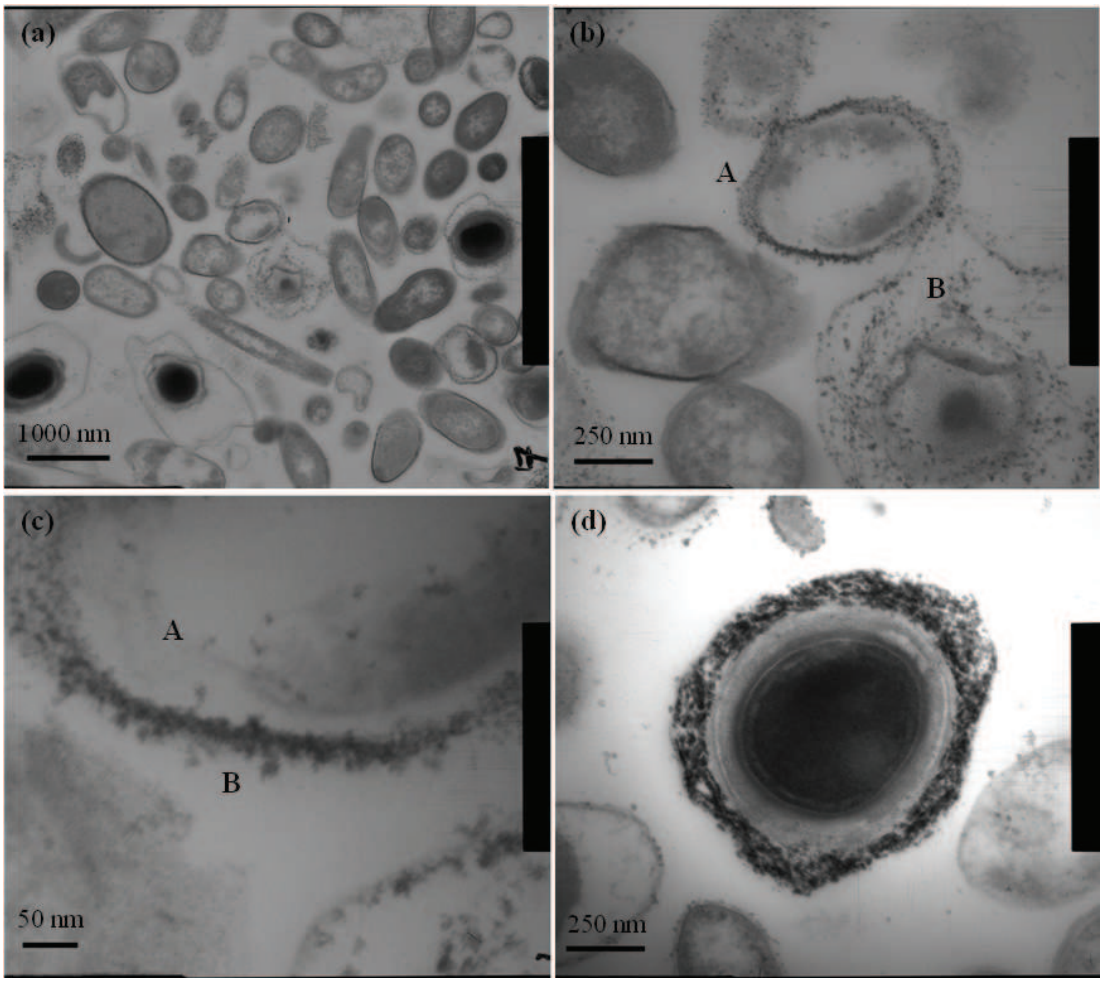

Figure 10.7. Transmission electron micrographs of mixed anaerobic microbial cultures exposed to phloroglucinol at 15000× magnification (a), 60000× magnification (b & d) and 200000× magnification (c). Figure 10.7(a) shows densely packed microorganisms with many spore-like cells. Figure 10.7(b) shows a disrupted outer membrane structure (A) and the coagulation of exopolysaccharides (B). Figure 10.7(c) shows fine details of crenation or separation of the cytoplasmic membrane from the cell envelope (A) and 'blebbing' (B). Figure 10.7(d) shows details of an endospore.

Morphological changes shown in Figures 10.8 and 10.9 suggest strong extra- and intra-cellular effects induced by cell exposure to phlorotannins. Figure 10.8a is a field view of bacterial cells showing extensively disturbed cell morphology (A), but also some apparently intact cells (B). From Figure 10.8b, clear evidence of cell disturbance can be observed with what appears to be cells fused together as a result of

membrane disturbance. Figure 10.8(c) shows details of lamina structures (A) with apparent cell membrane fusion.

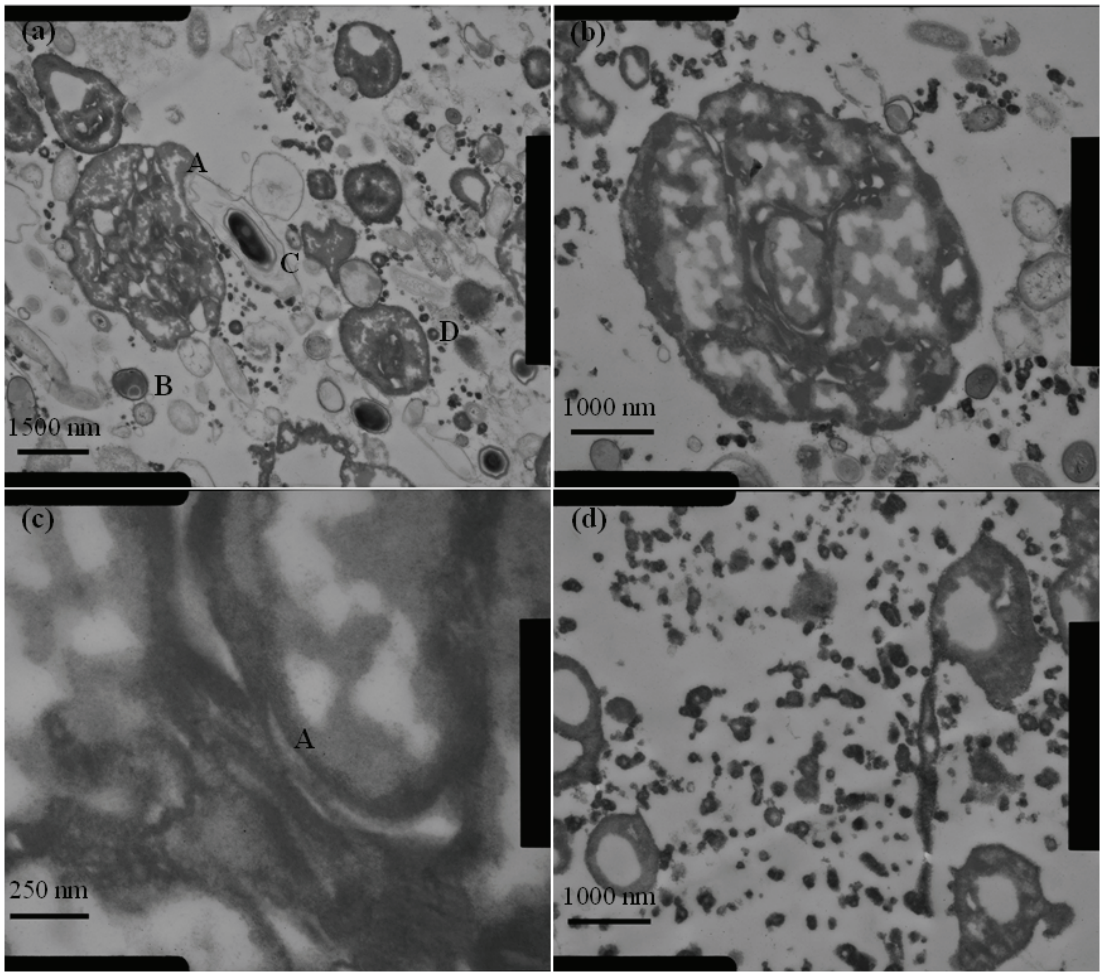

Figure 10.8. Transmission electron micrographs of mixed anaerobic microbial cultures exposed to phlorotannin at 7500× magnification (a), 15000× magnification (b & d) and 60000× magnification (c). Figure 10.8(a) shows affected cells (A), apparently intact cells (B), spore-like cells (C) and crenated cells (D). Figure 10.8(b) shows cells fused together. Figure 10.8(c) shows fine details of lamina structures resulting from cell membrane fusion (A). Figure 10.8(d) shows debris of cell components.

Debris of what seems to be cell components coagulated with phlorotannins observed visually and mentioned above during leakage assays can be noticed from most of the images with fine details shown in Figure 10.8d. Figure 10.9a shows evidence of crenation (A) and coagulation of cytosolic components (B).

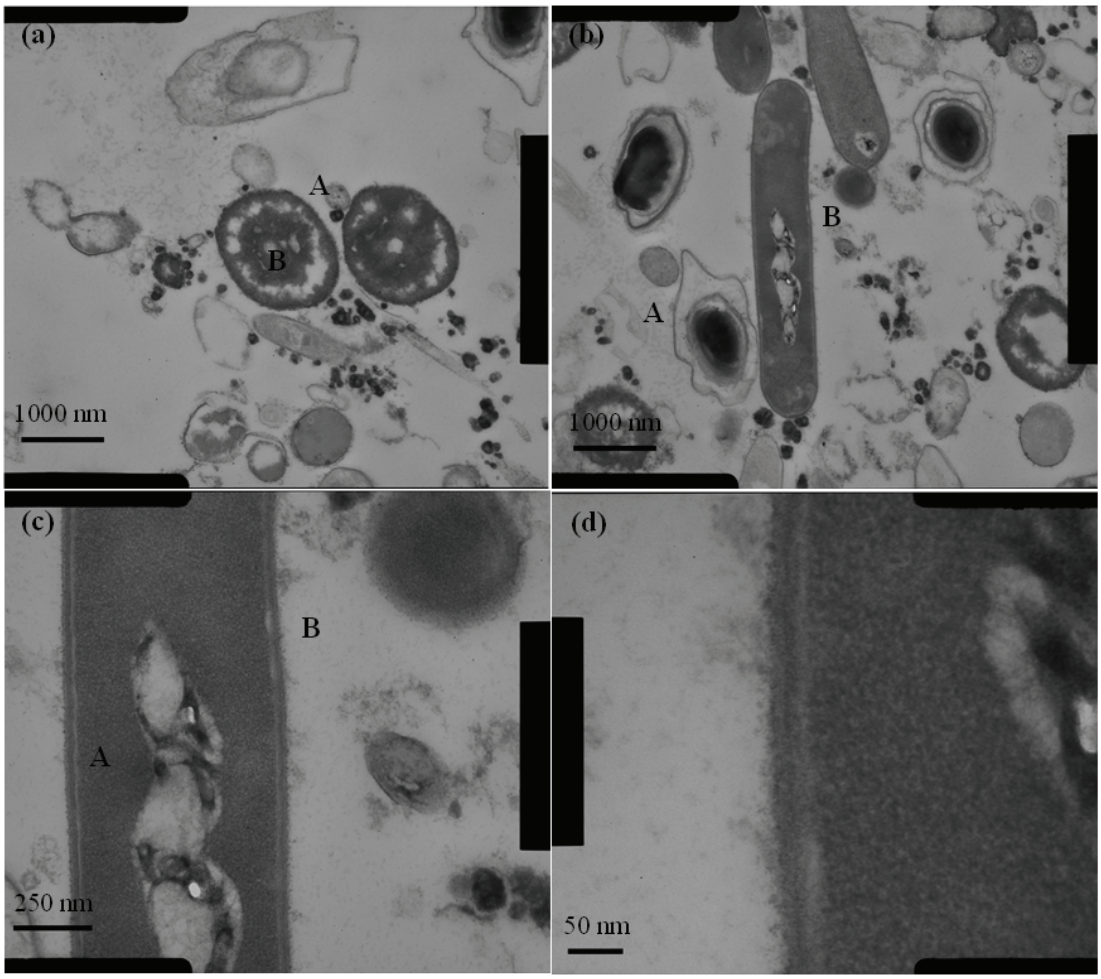

Figure 10.9. Transmission electron micrographs of mixed anaerobic microbial cultures exposed to phlorotannin at 15000× magnification (a & b), 60000× magnification (c) and 200000× magnification (d). Figure 10.9(a) shows two crenated cells (A) and the arrested early stage development of endospores (coagulated nucleic acids) (B). Figure 10.9(b) shows spore like cells (A) with a cell arrested at the early stage of sporulation (B). Figure 10.9(c) shows DNA supercoiling (A) and irregular cell membrane structure. Figure 10.9(d) shows fine details of a cell membrane with electron dense precipitated deposits.

Similarly to the observation made after the exposure of cells to phloroglucinol, Figure 10.9b shows spore-like structures (A) and a cell arrested at the early stage of the sporulation process (B). Figure 10.9c shows a higher magnification of a sporulating cell with bacterial DNA supercoiling (A) and an irregular cell envelope (B) as confirmed in Figure 10.9d where electron dense precipitated deposits can be seen on the surface of the cell membrane.

Transmission electron microscopy results suggest that disturbance of cell envelope may be a key step associated with the bactericidal action of phlorotannins and similar effects have been reported by Wang *et al.* (2009) when studying the sensitivity of *Escherichia coli* to phlorotannins. However, cell membrane disturbance could not be observed through 260 nm absorbing material leakage presumably due to the coagulation and sedimentation of phlorotannins with cell components that prevents their measurements with spectrophotometry. Final methane production (Figure 10.1b) and volatile fatty acids levels (Figure 10.2b) suggest that, whilst strong cellular effects have been induced by phlorotannins on most of the cells, some microorganisms proved less affected, as no intermediate components accumulated during the experiment and methanogenesis occurred at all phlorotannin levels tested. The slight decrease of methane production associated with the increase of phlorotannin concentration tends to show that a higher fraction of organic matter was consumed for biomass synthesis under unfavourable conditions and cell morphological changes observed by microscopy. From batch tests results (Figure 10.1) and microscopic observations (Figure 10.8) it appeared that phlorotannins induce a stronger inhibition on microorganisms than phloroglucinol (non-polymerised monomer of phlorotannin), which effect was overcome after a few days. Thus, the bactericidal activity of phlorotannins seems to be a function of the level of polymerisation of the compounds, which is in accordance with findings from Nagayama *et al.* (2002) who observed bactericidal activity of both crude and purified phlorotannins against pathogenic bacteria with the exception of phloroglucinol. Other phenolic compounds such as polymeric biguanides and particularly polyhexamethylene biguanides (PHMB) used for their bactericidal properties have been characterised, such as their activity increases on an increasing molecular weight

basis with increasing levels of polymerisation linked to enhanced inner membrane perturbation (Gilbert *et al.* 1990). Fixation of phlorotannins with small amounts of formaldehyde has been found to stimulate the anaerobic digestion of *Ascophyllum nodosum* (Moen *et al.* 1997), but its feasibility at large scale might be unrealistic.

10.4. Conclusions

This study has identified some of the effect of phlorotannins on mixed microbial cultures that can be found in anaerobic digesters. Through electron microscopy observations, it has been shown that phlorotannins induce significant extra- and intracellular effects upon cells, with the disruption of cell membranes observed with most microorganisms. Results also suggest that the bactericidal activity of phlorotannin is a function of the level of its polymerisation. Phloroglucinol was found to cause a kinetic inhibition at the beginning of the biodegradation process, whilst phlorotannin had a residual impact on methane production. The use of brown seaweed as a potential sole and co-substrate for anaerobic digestion is thus, likely to be dependent on phlorotannin concentrations and their bactericidal effect on microorganisms. However, inhibitory concentrations will be a function of polymerisation levels and dominant microbial species found in anaerobic systems. Polyphenolic compounds found in brown seaweed are often mentioned as potential inhibitors of anaerobic systems, but this is the first study to posit a probable mode of action for the antimicrobial effect of phlorotannins on mixed anaerobic microbial cultures.

Chapter 11
Anaerobic co-digestion of brown seaweed

In this chapter, the co-digestion of seaweed (Laminaria digitata) with vegetable residues (Pisum sativum) is investigated using both experimental and modelling approaches. The experimental approach involved the use of a laboratory-scale anaerobic digester. Assays were conducted at a fixed ratio of substrates over a period of 50 days. The addition of seaweed resulted in a rapid decrease of biogas production associated with the accumulation of volatile fatty acids, thereby suggesting that seaweed encourages imbalance between acidogenic and methanogenic activities. The ADM1 was modified to include an uncompetitive function that considers the effect of phlorotannins on acetoclastic methanogens and the implementation of an alternative microbial population with an increased tolerance to phenolic compounds. The modified model was able to accurately reproduce experimental observations and can be used to predict changes in methanogens populations induced by phlorotannins.

11.1. Introduction

Vegetable residues are characterised by a high fraction of volatile solids, high percentages of moisture and biodegradability and are thus, considered as a suitable feedstock for anaerobic degradation (Gunaseelan 1997; Ward *et al.* 2008). However, the availability of these residues is often season-dependent and a secure source of

feedstock might not be guaranteed throughout the year. The co-digestion of an alternative source of organic matter could be a solution to guarantee the stable operation of anaerobic systems season by season (see section 2.2.5). The successful co-digestion of substrates with different characteristics relies on the determination of a suitable blend that could secure acceptable concentrations of toxic compounds, guarantee a constant total mass input into the system and consider the different degradation and process rates of each substrate. To this extent, brown seaweed is considered as a suitable feedstock because of its good biodegradability and prevalence on Scottish coasts. However, seasonal fluctuations in the composition of seaweed associated with inherent inhibitory substances in the form of salts and polyphenolic compounds could impact negatively on the biogas conversion rate (see section 3.3). The effective anaerobic co-digestion of brown seaweed with milk (Matsui and Koike 2010) or by specifically adapting operational parameters (Chynoweth *et al.* 1981; Hanssen *et al.* 1987) has been reported in the literature. This study investigates the co-digestion of vegetable residues with brown seaweed using both experimental and modelling approaches. Process inhibition by phenolic compounds is implemented in a modified ADM1 as a reversible uncompetitive function considering the potential adaptation of microorganisms and the development of an alternative population of acetoclastic methanogens. Experimental assays were conducted in a one-stage anaerobic reactor and the results were used to calibrate the model parameters.

11.2. Methodology

11.2.1. Reactor studies

The reactor system used in this study is described in section 6.1.1. Feeding was carried out once daily and the reactor was operated under mesophilic temperatures

(37°C±1°C) with a 20 days hydraulic retention time (HRT). The reactor was firstly inoculated with anaerobically digested sludge (section 6.4) (20 g/l TS) and set in batch mode until the start-up of biogas production. *Laminaria digitata* and commercially available green peas were used as substrates and prepared accordingly to the methodology introduced in section 6.4. The dried substrates were added to represent a weight ratio of 10% and 90% for seaweed and peas respectively and diluted in 300 ml of tapwater to obtain a reactor total mass input of 2.7 kg $TS.m^{-3}.day^{-1}$ calculated by Equation 6.7. The characteristics of both substrates reported as representing the total mass input when considered individually can be found in Table 11.1.

Table 11.1. Characteristics of the substrates.

Parameter	Dry peas in tapwater (*Pisum sativum)*	Seaweed in tapwater (*Laminaria digitata)*
COD_{total} ($gCOD/m^3$)	48403.0	42127.0
$COD_{soluble}$ ($gCOD/m^3$)	25975.0	22438.0
VFA_{total} ($gCOD/m^3$)	653.5	514.3
TC (gC/m^3)	864.0	708.0
TOC (gC/m^3)	852.0	691.0
NH_4-N (g/m^3)	41.1	29.0
T.Phosphorous (g/m^3)	61.2	68.2
T.Alkalinity ($gCaCO_3/m^3$)	600.0	200.0
TS (g/l)	43.0	77.9
VS (%TS)	97.7	83.1
Ash (%)	2.3	20.5
C (% dry weight)	42.2	33.5
H (% dry weight)	6.4	5.1
N (% dry weight)	5.5	2.5
O (% dry weight)	42.8	37.0
S (% dry weight)	0.8	1.3
C:N ratio	8:1	13:1

The C, H, N and S contents of the substrates were determined in duplicate using a Perkin Elmer 2400 Series II CHNS Analyser (conducted by the University of Strathclyde, UK). The relative percentage of each compound was determined and the oxygen content was calculated by difference and corrected for ash. Figure 11.1 shows the time-line progression of the study. Vegetable residues were the only source of substrate until steady state was reached and for the first 20 days of experiment.

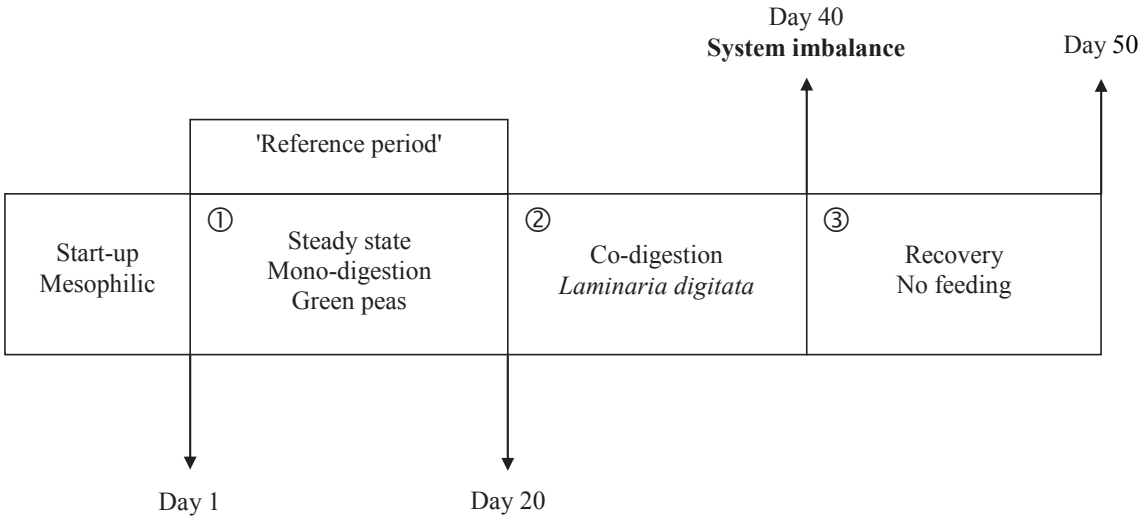

Figure 11.1. Time-line progression of the co-digestion study.

During the experiment, sodium levels were kept below 600 mg/l by tapwater dilution and the influent COD/SO_4^- ratio at about 15. This was to ensure negligible effect of salt on microorganisms (see Chapter 8) and favour methanogens over sulphate-reducing bacteria. The latter being usually favoured at COD/SO_4^- ratios lower than 5 (Briones *et al.* 2007). The phlorotannin content of the seaweed was found equal to about 1% of total weight and measured according to the methodology described in section 6.2.12.

11.2.2. Model modifications

Fezzani and Ben Cheikh (2009) successfully added the biodegradation of phenolic compounds in the ADM1 original structure through disintegration, hydrolysis, uptake and decay of phenol biomass. However, the authors observed that the addition of these specific processes in the model would significantly increase its complexity and even resulted in less accurate predictions for pH. Moreover, their addition is likely to be unnecessary when the total concentration of phenols reported in seaweed is usually much lower when compared to those found during the treatment of olive mill wastes. Therefore, the implementation of phenolic compounds as a specific class of composite, particulate and soluble substrates is not considered in this work. Since the potential inhibition resulting from phlorotannins is a result of the addition of seaweed in the anaerobic system, it is proposed to add an extra inhibition dependent of the seaweed concentration in the system inflow. This approach is supported by the fact that the antibacterial activity of phlorotannin has been found dependent on polymerisation levels (section 10.3.3) and both identification and quantification of phlorotannins levels are made difficult by the lack of commercially available standards. The characteristics of both substrates are loaded separately in the model and their concentration calculated according to their respective flow rates, as shown in Figure 11.2. The combination module sums up the characteristics from both substrates and combines them proportionally to their flow rate. It is therefore possible to dynamically change the ratio of substrates fed into the reactor.

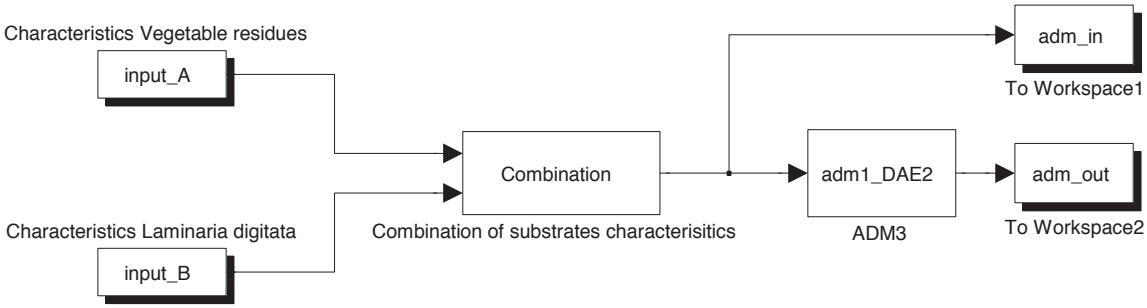

Figure 11.2. Substrates co-digestion module implemented in Simulink.

The updated version of the ADM1 biological reactions matrix can be found in Table 11.2 using the nomenclature introduced in Chapter 4 (Tables 4.2 and 4.3). Based on the results obtained in Chapter 10 and owing that acetoclastic methanogens are generally more sensitive than hydrogenotrophs in the presence of phenolic compounds at inhibitory concentrations (Kim *et al.* 1996), the inhibitory function will consider exclusively acetate degrading microorganisms. The effect of inhibitory concentrations of phlorotannins on microorganisms can be described as a biostatic inhibition, i.e. normally reversible inhibition disrupting homeostatis and influencing kinetic uptake and growth (Batstone *et al.* 2002). The inhibitory function implemented in this work relies on phlorotannin concentrations and the uptake of acetate is therefore described by two different functions. When no seaweed (and hence, no phlorotannins) is introduced in the system, the uptake of acetate follows the original equation implemented in the ADM1. In the case of co-digestion, extra-inhibition factors integrated to the uptake equation are added (Table 11.2).

Table 11.2. Modified ADM1 biological reactions matrix.

Component →	i	7	9	10	11	13	22	22'	Rate (ρ_j, kg COD.m^{-3}.d^{-1})	
j	Process ↓	S_{ac}	S_{ch4}	S_{IC}	S_{IN}	X_c	X_{ac}	$X_{ac,Lam}$	*Mono-digestion*	*Co-digestion Laminaria*
11	Uptake of acetate	-1	$(1-Y_{ac})$	$-\sum_{i=1-9,11-24} C_i \nu_{i,11}$	$-(Y_{ac}).N_{bac}$		Y_{ac}		$k_{m,ac} \cdot \frac{S_{ac}}{K_{Sac} + S_{ac}} \cdot X_{ac} \cdot I_3$	$\frac{k_{m,ac} \cdot X_{ac} \cdot S_{ac}}{K_{Sac} + S_{ac} \cdot \left[1 + \frac{K_{I,lam}}{S_{lam}}\right]} \cdot I_3$
11'	Uptake of acetate by tolerant methanogens	-1	$(1-Y_{ac,lam})$	$-\sum_{i=1-9,11-24} C_i \nu_{i,11}$	$-(Y_{ac,lam}).N_{bac}$			$Y_{ac,lam}$	$k_{m,ac,lam} \cdot \frac{S_{ac}}{K_{Sac,lam} + S_{ac}} \cdot X_{ac,lam} \cdot I_3$	$\frac{k_{m,ac,lam} \cdot X_{ac,lam} \cdot S_{ac}}{K_{Sac,lam} + S_{ac} \cdot \left[1 + \frac{K_{I,lam}}{S_{lam}}\right]} \cdot I_3$
18	Decay of X_{ac}					1	-1		$k_{dec,Xac} \cdot X_{ac} \cdot I_{dec,Xac}$	
18'	Decay of $X_{ac,lam}$					1		-1	$k_{dec,Xac,lam} \cdot X_{ac,lam} \cdot I_{dec,Xac,lam}$	
		Total acetate (kg COD.m^{-3})	Methange gas (kg COD.m^{-3})	Inorganic carbon (kmoleC.m^{-3})	Inorganic nitrogen (kmoleN.m^{-3})	Composites (kg COD.m^{-3})	Acetate degraders (kg COD.m^{-3})	Tolerant acetate degraders (kg COD.m^{-3})	Inhibition factors: $I_3 = I_{pH} \cdot I_{IN,\lim} \cdot I_{NH_3}$ Decay factors: $I_{dec,Xac} = 1 + \frac{S_{lam}}{K_{I,lam,Xac}}$ $I_{dec,Xac,lam} = 1 + \frac{S_{lam}}{K_{I,lam,Xac,lam}}$	

Although inhibition by phlorotannins might impair methanogenesis and result in process failure, its effect has been found to depend on the morphology of microorganisms and it is believed that the development of a resistant microbial consortium is possible and has been confirmed by batch studies (section 10.3.1) and microscopic observations (section 10.3.3). This increased tolerance is believed to occur through a complete shift in the species nature of the microbial population. Thus, it is possible to consider two different acetic acid-utilising species with a different degree of tolerance to phlorotannins. The toxicity of phlorotannins is introduced by a decay factor depending on the seaweed influent concentration. The decay rate is increasing with the increase of phlorotannin concentration, but has two different rates defined by $I_{dec,Xac}$ or $I_{dec,Xac,lam}$ (Table 11.2) depending on the microbial group on which it is applied, i.e. tolerant or non-tolerant. The two microbial groups are characterised by different decay and uptake rates, half-saturation constants and yields relative to their respective tolerance.

11.3. Results and discussions

11.3.1. Parameters optimisation

The influent composition was determined separately for both substrates since the updated model combines their characteristics in a subsequent step. Hence, it is possible to change their ratio in the influent without estimating the model parameters every time. The transformer model elaborated by Zaher *et al.* (2009a) was used to obtain the ADM1 input from the substrates characteristics determined in Table 11.1. First order parameters corresponding to the hydrolysis kinetic rates of carbohydrates, lipids and proteins were set at rates similar to the disintegration step and determined through preliminary biodegradation batch tests. Since initial conditions were not

determined, the initial simulation period was not taken into account and outputs were considered only after the model reached 1000 days of simulation time corresponding to the numerical stabilisation of the system. Firstly, newly introduced parameters were estimated by fitting the model output to experimental values. Once the model exhibited acceptable results, parameter values were further refined by using the Levenberg-Marquardt iterative method, as described in section 6.6, through 88 model calibration runs. Estimates and standard deviations for newly introduced parameters, as calculated from the Jacobian matrix, can be found in Table 11.3.

Table 11.3. Parameters estimation and standard deviations.

Parameter	Unit	Definition	Value	Standard deviation	Error in %
k_dec_Xac_lam	d^{-1}	First order decay rate of phlorotannin tolerant acetate degraders	0.01	4.81E-02	375.43
K_S_ac_lam	kg COD·m^3	Half saturation constant of acetate uptake by phlorotannin tolerant acetate degraders	0.63	3.83E-02	6.08
Y_ac_lam	-	Yield of phlorotannin tolerant acetate degraders on acetate	0.32	1.83E-03	5.73E-01
k_I_lam	kmole·m^3	Inhibition constant to acetate uptake	0.22	1.47E-05	6.62E-03
k_I_lam_Xac_lam	kmole·m^3	Inhibition constant to phlorotannin tolerant acetate degraders decay	0.10	1.47E-05	1.47E-02
k_I_lam_Xac	kmole·m^3	Inhibition constant to normal acetate degraders decay	0.05	1.47E-05	0.03
k_m_ac_lam	d^{-1}	Monod specific uptake rate of acetate by phlorotannin tolerant acetate degraders	12.00	2.08E-02	0.17

The statistical confidence on the parameters estimates is relatively high since standard deviations and thus, errors calculated are low. Only the parameters $k_{dec,xac,lam}$ and $k_{S,ac,lam}$, corresponding to the decay rate of phlorotannin-tolerant acetate degraders and their half saturation constant respectively, could not be estimated with sufficient accuracy. A sensitivity analysis on the newly introduced parameters was conducted following the method of Morris introduced in section 7.2.2 and according to the

methodology shown on Figure 7.1. Parameters considered for the analysis, their range of variation and their probability distribution functions are reported in Table 11.4. The output of interest selected for the analysis was the model biogas flow rate (Q_{gas}). The parameter K_{I_Lam} representing the inhibitory concentration of phlorotannin and used during the calculation of the uncompetitive inhibition is not considered in this analysis since only parameters relevant to the tolerant microbial population are of interest when the process is inhibited. Results from the sensitivity analysis can be seen in Figure 11.3. The method of Morris shows that three parameters are particularly influential, namely $K_{S\_ac\_Lam}$, Y_{ac_Lam} and $K_{dec\_xac\_Lam}$.

Table 11.4. Newly introduced parameters and values for sensitivity analysis.

Parameter	Range	Units	PDF
$K_{dec\_Xac\_Lam}$	0.005-0.03	d^{-1}	U
$K_{I\_Lam\_Xac\_Lam}$	1E-05-1E-01	$kgCOD/m^3$	U
$K_{I\_Lam\_Xac}$	1E-05-1E-01	$kgCOD/m^3$	U
$K_{m\_ac\_Lam}$	7-12	d^{-1}	U
$K_{s\_ac\_Lam}$	0.1-0.7	$kgCOD/m^3$	U
Y_{ac_Lam}	0.03-0.1	$kgCOD_X/kgCOD_S$	U

All three parameters are linked with the newly introduced population of acetate degraders and represent their half saturation, the yield of tolerant acetate degraders on acetate, and their first order decay rate respectively. These parameters have direct influence upon the population of tolerant acetate utilisers and hence, require careful calibration. The maximum specific uptake rate of acetate by adapted degraders ($K_{m\_ac\_Lam}$), together with the decay factors of phlorotannin-tolerant acetate degraders ($K_{I\_Lam\_xac\_lam}$) and normal acetate degraders ($K_{I\_Lam\_xac}$) are set to default values in order for the conditions $K_{m\_ac} < K_{m\_ac\_Lam}$ and $K_{I\_Lam\_xac} < K_{I\_Lam\_xac\_lam}$ to be fulfilled.

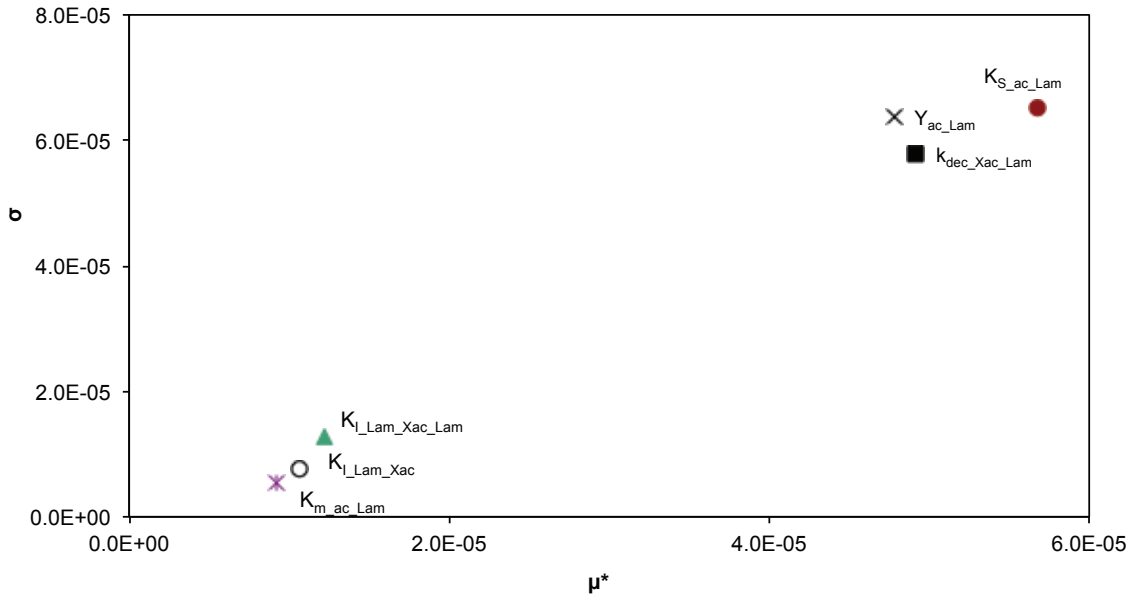

Figure 11.3. Morris sensitivity analysis for the newly introduced parameters.

Parameters, such as particulate components, half saturation values or decay rates were taken to be equal to the values suggested by Batstone *et al.* (2002) and from the Matlab implementation of Rosen *et al.* (2006). Attempts to fit competitive and non-competitive inhibition functions were performed, but no convergence was found.

11.3.2. Simulation of reactor performance

Experimental and simulated values of gas flow rate can be seen in Figure 11.4. The model was able to accurately simulate the production of biogas for the reference period of the assay. At the start of co-digestion (Day 21), a slight decrease of biogas production was observed and the model was able to represent this decrease with deviations of about 6 to 15% between measured and simulated values. These discrepancies might be explained by the non-calibration of some parameters, such as the gas-liquid transfer coefficient and gas solubility coefficients (see section 8.3.2).

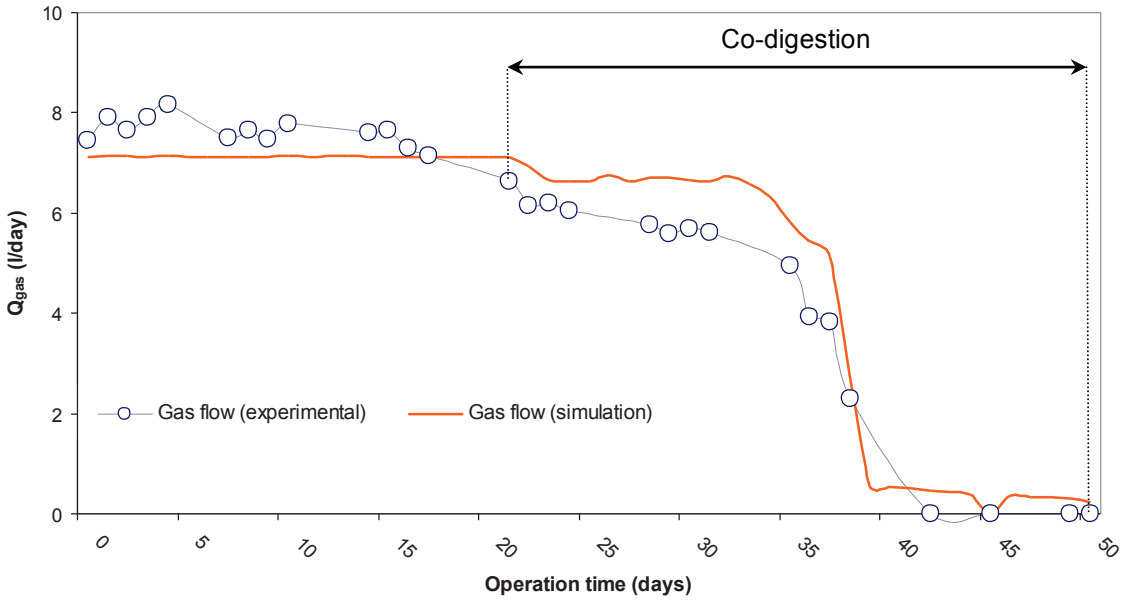

Figure 11.4. Gas flow rate simulation and experimental values.

However, the calibrated model was able to accurately predict system failure at Day 39. As observed experimentally, pH values rapidly decreased after the addition of seaweed in the digester. Simulation values for pH are in good agreement with measurements from the beginning of the experiment to Day 29, as shown in Figure 11.5. During co-digestion, the model was able to reproduce the general trend of pH decrease. Fezzani and Ben Cheikh (2009) included the effect of phenolic compounds on pH by adding extra parameters in the charge balance (Equation 4.7). However, they reported that this addition resulted in less accurate predictions for the effluent pH when compared to the original model.

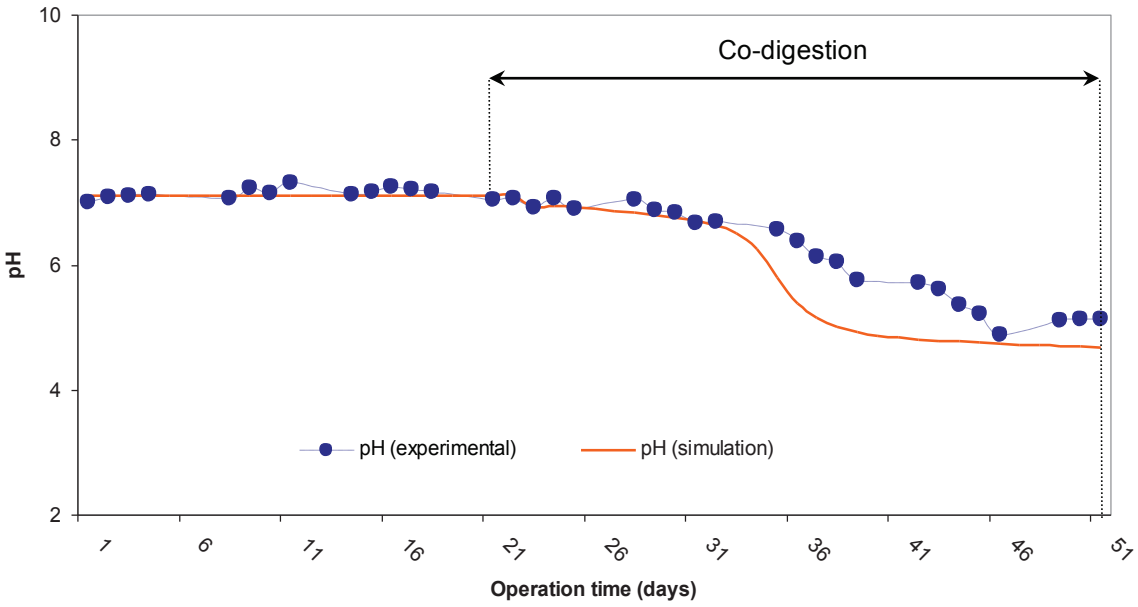

Figure 11.5. pH simulation and experimental values.

A possible reason for pH underestimation is the simultaneous overestimation of VFA as shown on Figure 11.6.

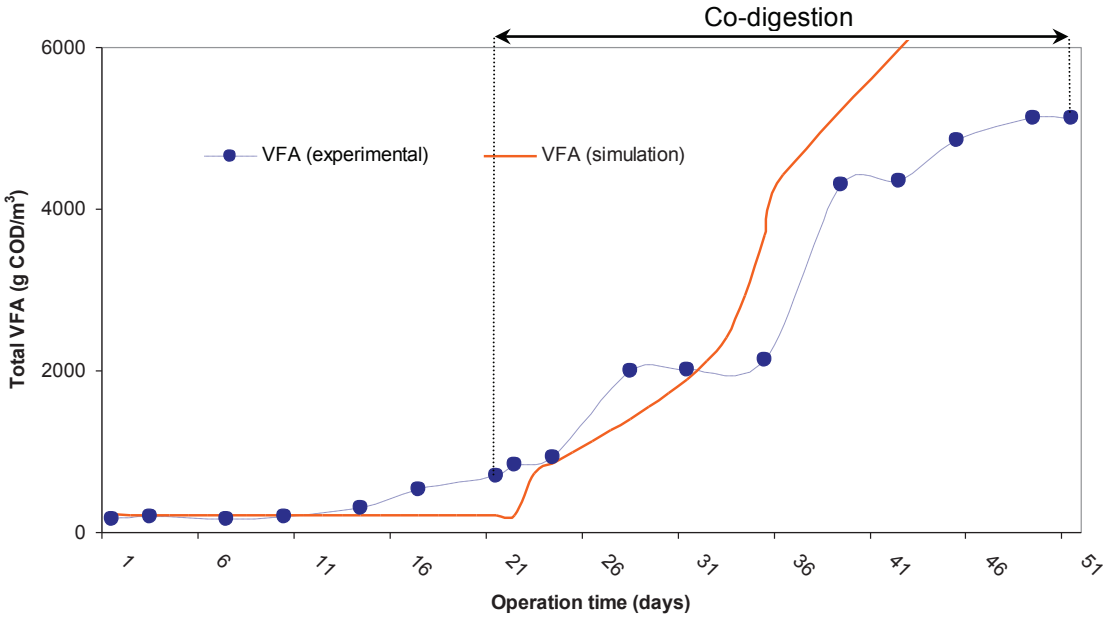

Figure 11.6. VFA simulation and experimental values.

At steady state (day 1 to 20), the model correctly predicted the total volatile fatty acid concentration. Inconsistencies observed between experimental values and simulations suggest that some of the parameters, such as kinetic rates, could be calibrated more precisely by measuring individual concentrations of VFA. The simulation of acetoclastic methanogens populations shown that normal acetoclastic methanogens levels rapidly decrease since being negatively impacted by the introduction of phlorotannins, whilst tolerant methanogens remain unaffected. During both stable conditions and co-digestion, the calculation and analysis of standardised residuals was found to follow a normal distribution for the biogas flow rate, pH and VFA concentrations according to the Shapiro-Wilk test at a 5% level of significance with determination coefficients (r^2) equal to 0.84, 0.93 and 0.92 respectively, hence supporting the validity of the modelling approach used in this work.

Sodium levels were kept below inhibitory concentrations and methanogens were believed to be favoured over sulphate-reducing bacteria throughout the assay. Moreover, alkalinity levels were found to increase from 1500 mg $CaCO_3$/l to over 3000 mg $CaCO_3$/l when co-digestion started, hence supporting the fact that pH drop is a consequence of VFA increase which itself results from methanogenesis inhibition. Sulphide concentrations remained stable for the whole duration of the study and under inhibitory concentrations, whereas ammonia nitrogen levels were monitored through the measurement of ammonium and estimated by Equation 6.6 with concentrations found to be below 100 mg/l as NH_3. At the start of co-digestion, a slight decrease of biogas production was expected since *Laminaria digitata* is characterised by a smaller fraction of volatile solids than green peas (Table 11.1) which, at a constant total mass input, would result in a smaller fraction of potentially biodegradable organic matter. In the light of the measured parameters, the strong system imbalance that resulted in a

rapid decrease of biogas production is most likely a result from the inhibitory effect of phlorotannin on methanogens as observed in Chapter 10. Phlorotannin levels were estimated to a maximum of 40 mg/l, but their inhibitory effect combined with a possible microorganisms washout due to low hydraulic retention time might have impaired microbial adaptation and resulted in complete process failure after pH dropped below 6.5. The optimum carbon to nitrogen ratio for anaerobic digestion is often reported around 25:1, but anaerobic systems will operate successfully at ratios as low as 10:1. During the anaerobic digestion of brown seaweed, Chynoweth *et al.* (1987) found that the production of biogas was highest at low C:N ratios of about 14:1 and decreased when the carbon to nitrogen ratio reached 24:1. The relatively low C:N ratio found for green peas (Table 11.1) was increased by the addition of seaweed but was not found to impact positively on biogas production.

11.3.3. Further modelling considerations

In the ADM1, disintegration and hydrolysis processes are described by first-order kinetics (section 4.2.3) and process rates are usually determined experimentally through batch tests or reactor studies. Hence, the ADM1 can be used to model co-digestion at fixed ratio since disintegration and hydrolysis rates will be determined experimentally for a specific substrate ratio. However, varying concentrations of these substrates will ineluctably impact on hydrolysis rates, and are likely to impair the accuracy of the model. To this extent, Zaher *et al.* (2009b) developed a '*combiner*' model separating the hydrolysis process for all substrates streams according to their flow rate and combining the results for the subsequent steps of anaerobic degradation. Hence, the original model structure can be modified to include the effect of sodium ions and biomass adaptation to salinity, as investigated in Chapters 8 and 9, along

with the addition of an alternative population of acetic acid utilisers to consider the inhibitory effect of phlorotannin. The dynamic change of substrate ratio and varying hydrolysis rates should also be considered. The schematic representation of such a model as implemented in Simulink is shown on Figure 11.7. Inputs characterising the treated substrates are uploaded in the model (❶) and parameters are used for the calculation of hydrolysis with their respective rates (❷). The hydrolysis modules only take into account disintegration and hydrolysis steps by considering null uptake and decay rates for the subsequent processes with gas-liquid transfer coefficients taken equal to zero. First order parameters corresponding to disintegration and hydrolysis rates and specific yield rates are adjusted separately for each substrate. The concentrations of both sodium and phlorotannin in the system are calculated according to their specific concentrations in each substrate (❸). Results from sodium calculation and hydrolysis are rearranged and combined (❹) before being calculated in a modified version of the model where all parameters for disintegration and hydrolysis are set to zero and only acidogenesis, acetogenesis and methanogenesis steps are active (❺). Hydrogen and pH are calculated only once in the model and linked to hydrolysis modules (❻). Two populations of acetoclastic methanogens are introduced to consider the uncompetitive inhibition used to represent the effect of phlorotannins on microorganisms.

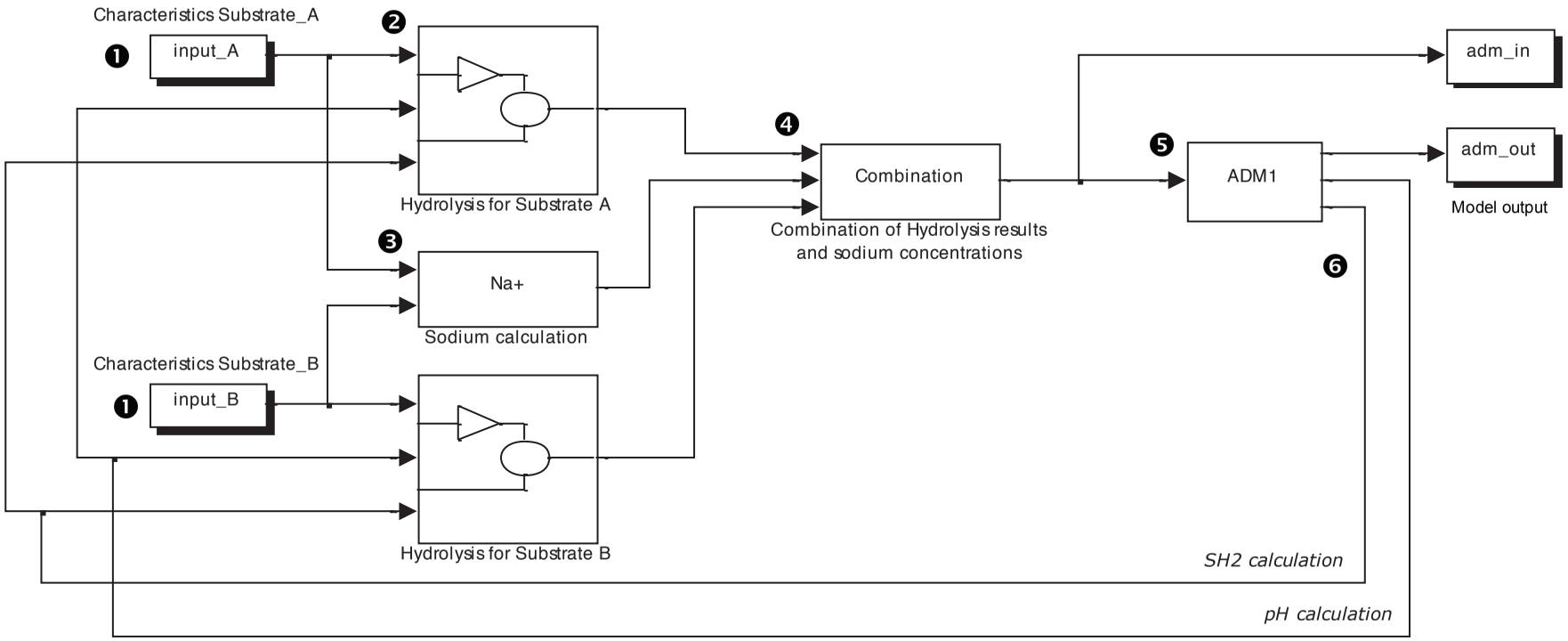

Figure 11.7. Co-digestion model with sodium calculation and separate hydrolysis rates as implemented in Simulink.

11.4. Conclusions

Both the experimental and modelling approaches used in this study have shown that the co-digestion of *Laminaria digitata* with vegetable residues at a weight ratio of 10% seaweed in the total mass input, resulted in system imbalance and inhibition of methanogenesis, probably triggered by phlorotannin toxicity, leading to a rapid decrease in biogas production and simultaneous accumulation of volatile fatty acids. The ADM1 has been modified and calibrated to take into account the effect of phlorotannins on acetate degrading organisms. The introduction of an uncompetitive function to the rate of acetate uptake has been found appropriate and led to the accurate simulation of biogas flow rate, pH and VFA. With the addition of an alternative population of acetoclastic methanogens, the higher tolerance of some microorganisms to phlorotannins can be successfully simulated. The adjustment of operational parameters, such as the effective hydraulic retention time and a gradual increase of seaweed quantities fed into the system can be used to favour the adaptation of microorganisms to phlorotannins and hence, improve process stability during the anaerobic co-digestion of brown seaweed.

Chapter 12
Optimisation of co-digestion of brown seaweed

This chapter focuses on the optimisation of co-digestion of brown seaweed (Laminaria digitata) and vegetable residues (Pisum sativum). Experimental studies consisted in the co-digestion of both substrates at different loading rates using a laboratory-scale anaerobic digester. Results suggest that whilst the addition of seaweed at high loading rate can impact negatively on process stability, a gradual addition of Laminaria digitata at low loading rate together with an increase in buffering capacity of the system can enhance biogas production.

12.1. Introduction

Carbohydrate-rich substrates, such as vegetable residues, are easily transformed to volatile fatty acids (VFA) in anaerobic systems (Jiang *et al.* 2012). Among strategies to improve system stability and enhance biogas yields, co-digestion has been increasingly used in the past few years (Mata-Alvarez *et al.* 2009) and found to be successful in ensuring stable performances during the treatment of fruit and vegetable wastes (Ward *et al.* 2008). Co-digestion has also been reported to improve the buffering capacity of anaerobic systems and hence, reduce the impact of VFA accumulation (Akunna *et al*. 2007; Khalid *et al.* 2011). However, the addition of *Laminaria digitata* in a system treating vegetable residues has been found to be detrimental to the digestion process with the inhibition being attributed to the

antimicrobial effect of phenolic compounds characterising brown seaweed (see Chapter 11). Moen *et al.* (1997) also found that the dominant factor for the conversion of brown seaweed during anaerobic digestion was the presence of polyphenols. Yet, the adaptation of microorganisms to polyphenols has been found possible with methanogenesis occurring at high phlorotannin levels (see Chapter 10) and it is believed that the adjustment of operational parameters could enhance the anaerobic digestion of brown seaweed. In this work, co-digestion of *Laminaria digitata* and green peas is investigated at varying organic loading rates and weight ratios of the substrates to determine the operational conditions necessary for effective anaerobic degradation of both substrates.

12.2. Methodology

A single-stage anaerobic reactor was used for the study and is detailed in section 6.1.1. Feeding was carried out once daily and the reactor was operated under mesophilic temperatures (37°C±1°C) with a 20 days hydraulic retention time (HRT). The reactor was firstly inoculated with anaerobically digested sludge (as described in section 6.4) and set in batch mode until the start-up of biogas production. *Laminaria digitata* and commercially available green peas were used as substrates and prepared according to the methodology introduced in section 6.4. Varying weight ratios of each substrate were mixed with 300 ml of tapwater before addition to the reactor. Two distinct experiments were conducted and are detailed below.

12.2.1. High loading rate and reactor recovery

The first co-digestion study was conducted over 190 days involving 6 different stages following the start-up period. Figure 12.1 shows the time-line progression of the study

and the different stages. For the entire duration of this assay, seaweed represented 2% of the total organic loading rate (OLR). The phlorotannin content of the seaweed was about 1% of total weight. Sodium ion concentrations in the reactor were found to vary between 150 mg Na^+/l and 210 mg Na^+/l. After start-up, the reactor was fed exclusively with green peas at an OLR of 2.67 kg $VS.m^{-3}.day^{-1}$ for 15 days (①). Between Days 16 and 31, 2% by weight of the green peas was replaced with an equal amount of seaweed, whilst maintaining the overall OLR constant (②). The system was then operated until Day 66 in a sequence alternating feeding at a loading rate of 2.67 kg $VS.m^{-3}.day^{-1}$ and periods without feeding in an attempt to stabilise the system and limit the accumulation of volatile fatty acids (③). A similar strategy was used at loading rates between 0.89 and 2.67 kg $VS.m^{-3}.day^{-1}$ for an additional 29 days (④) in a further attempt to recover the process and maintain near neutral pH values. Between Days 95 and 155, green peas were solely added into the system following a pattern alternating feeding at an OLR between 0.89 and 1.78 kg $VS.m^{-3}.day^{-1}$ and periods without feeding in order to decrease VFA levels (⑤). In the last stage of the experiment, both substrates were added daily at a constant OLR of 0.19 kg $VS.m^{-3}.day^{-1}$ (⑥).

12.2.2. Reduced loading rate and increase of buffering capacity

A second co-digestion study was conducted at lower loading rates in order to prevent the rapid accumulation of volatile fatty acids and hence prevent the extensive variation of pH within the system. The experiment was conducted over 220 days involving 6 distinct stages following the start-up period. Figure 12.2 shows the time-line progression of the assay.

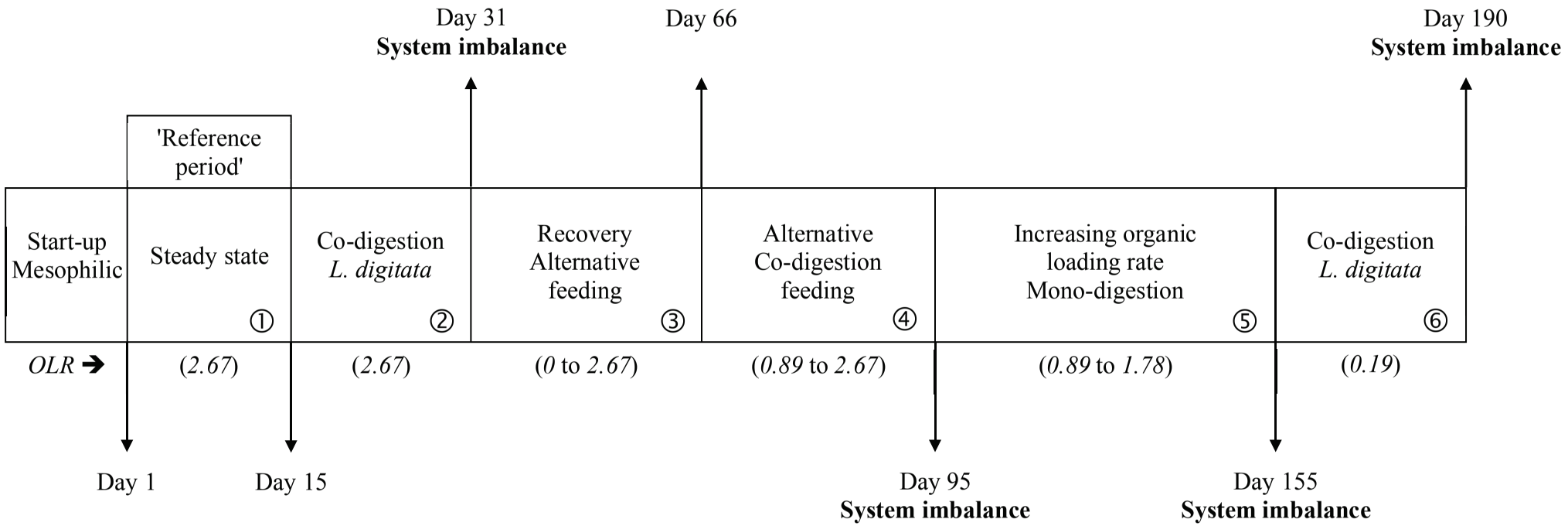

Figure 12.1. Time-line progression of the first co-digestion study (high loading rate). The values taken by the organic loading rate are shown in brackets under each stage of the experiment and expressed in kg $VS.m^{-3}.day^{-1}$.

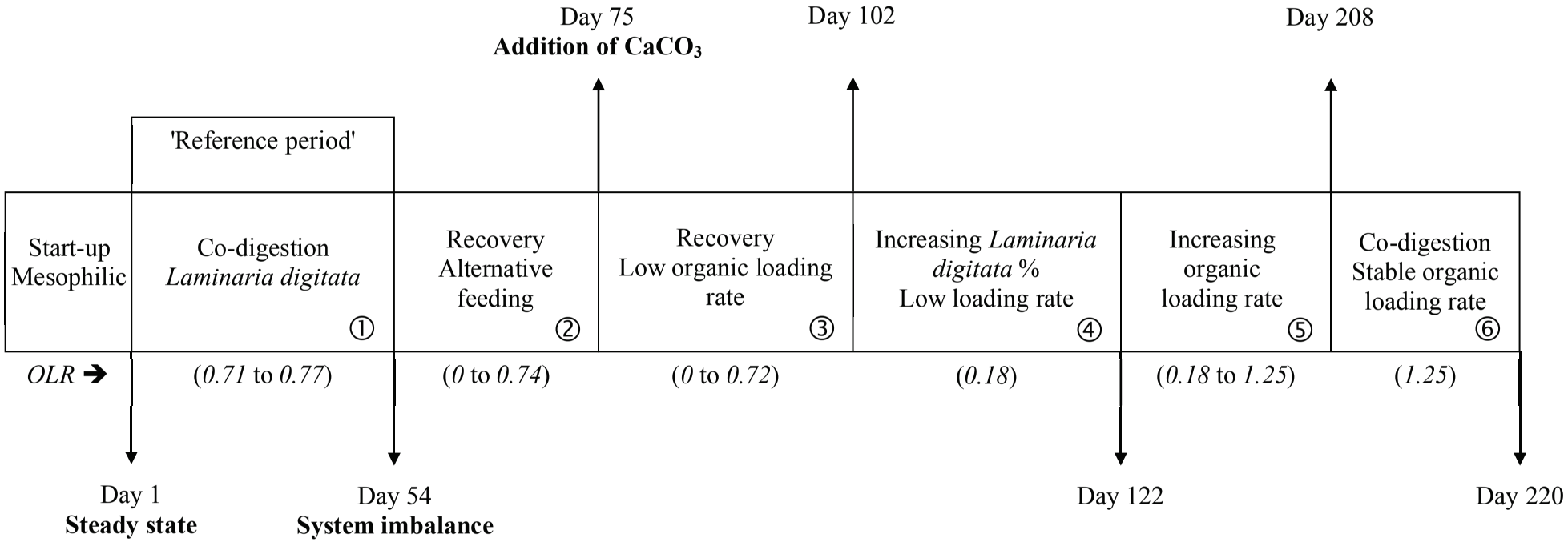

Figure 12.2. Time-line progression of the second co-digestion study (reduced loading rate). The values taken by the organic loading rate are shown in brackets under each stage of the experiment and expressed in kg $VS.m^{-3}.day^{-1}$.

The phlorotannin content of the seaweed represented 0.6% of total weight. The sodium concentration in the system was about 250 mg Na^+/l. After start-up, the reactor was fed exclusively with green peas for 10 days at an OLR of 0.71 kg $VS.m^3.day^{-1}$ and seaweed was gradually added to increase the loading rate to 0.77 kg $VS.m^{-3}.day^{-1}$ until Day 54 (①). Between Days 55 and 74, the reactor was operated with a lower fraction of seaweed in the influent feedstock in a sequence alternating feeding at constant loading rate and periods without feeding in an attempt to limit the accumulation of volatile fatty acids (②). On Day 75, calcium carbonate (16.5 g as $CaCO_3$) was introduced in the system in order to increase the system buffering capacity and from this stage forward, 0.1 g of $CaCO_3$ was added daily in the system to compensate for the low alkalinity characterising the dilution water used in the experiment. Between Days 88 to 102, the loading rate was decreased from 0.72 kg $VS.m^{-3}.day^{-1}$ to 0.18 kg $VS.m^{-3}.day^{-1}$ with seaweed representing less than 2% of the total OLR in an attempt to reduce VFA levels (③). The loading rate was then kept constant at 0.18 kg $VS.m^{-3}.day^{-1}$ for a further 20 days during which the ratio of seaweed was increased to represent up to 35% of the total OLR in order to further contribute to the decrease of volatile fatty acids levels (④). After the stabilisation of the system, the loading rate was gradually increased along with the percentage of brown seaweed for a period of 86 days (⑤) and the experiment was continued for a further 12 days at a steady OLR of 1.25 kg $VS.m^{-3}.day^{-1}$ during which intermediary indicators remained stable (⑥).

12.3. Results and discussion

12.3.1. System performances and recovery at high loading rate

Total biogas and methane production are shown in Figure 12.3 whilst variations in volatile fatty acids concentrations and pH can be seen in Figure 12.4. During the first phase of the assay (green peas only), the system produced a daily amount of biogas and methane of 8 and 5.5 litres respectively with pH values near to neutral. With the addition of seaweed, biogas yield and methane content immediately dropped with a corresponding increase and decrease of VFA and pH levels respectively. In an attempt to stabilise the system, feeding was interrupted but VFA concentrations continued to fluctuate resulting in a substantial drop in pH between Days 31 and 35 as shown in Figure 12.4.

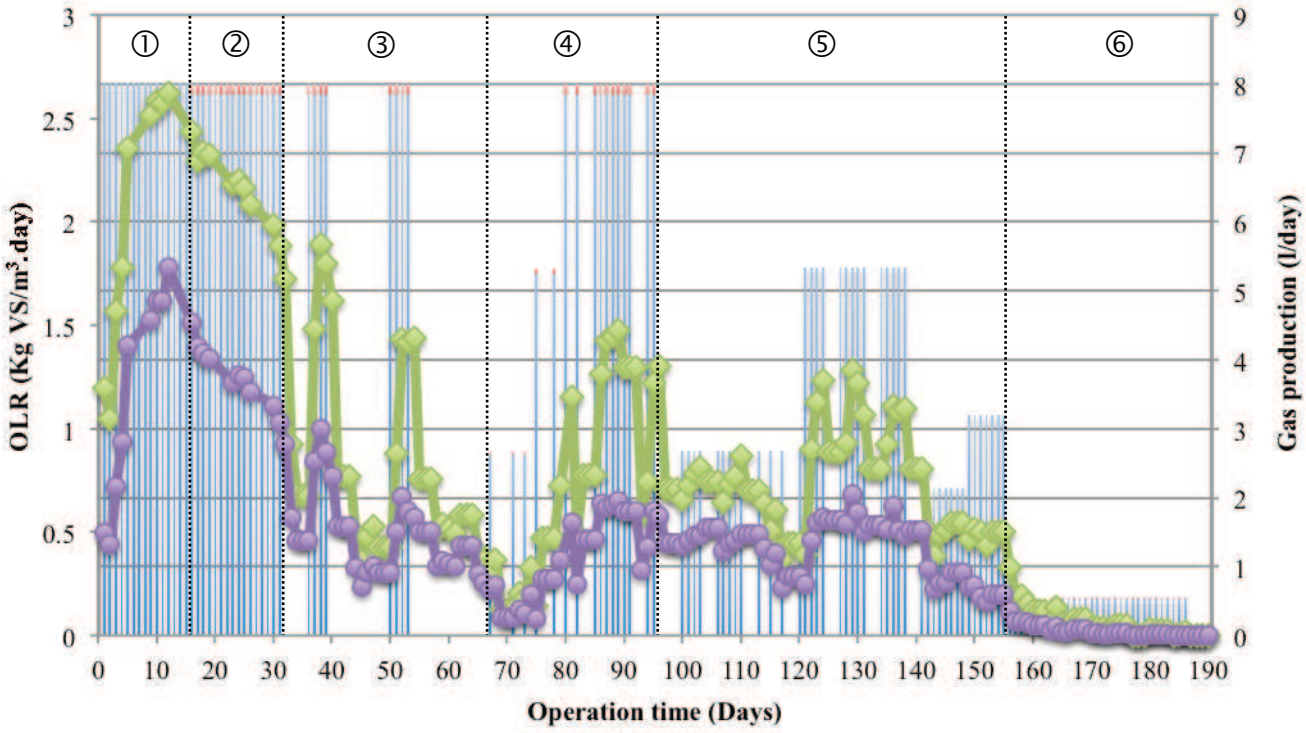

Figure 12.3. Biogas production: ◆ total; ● methane and OLR: ■ vegetable residues; ■ seaweed over 190 days.

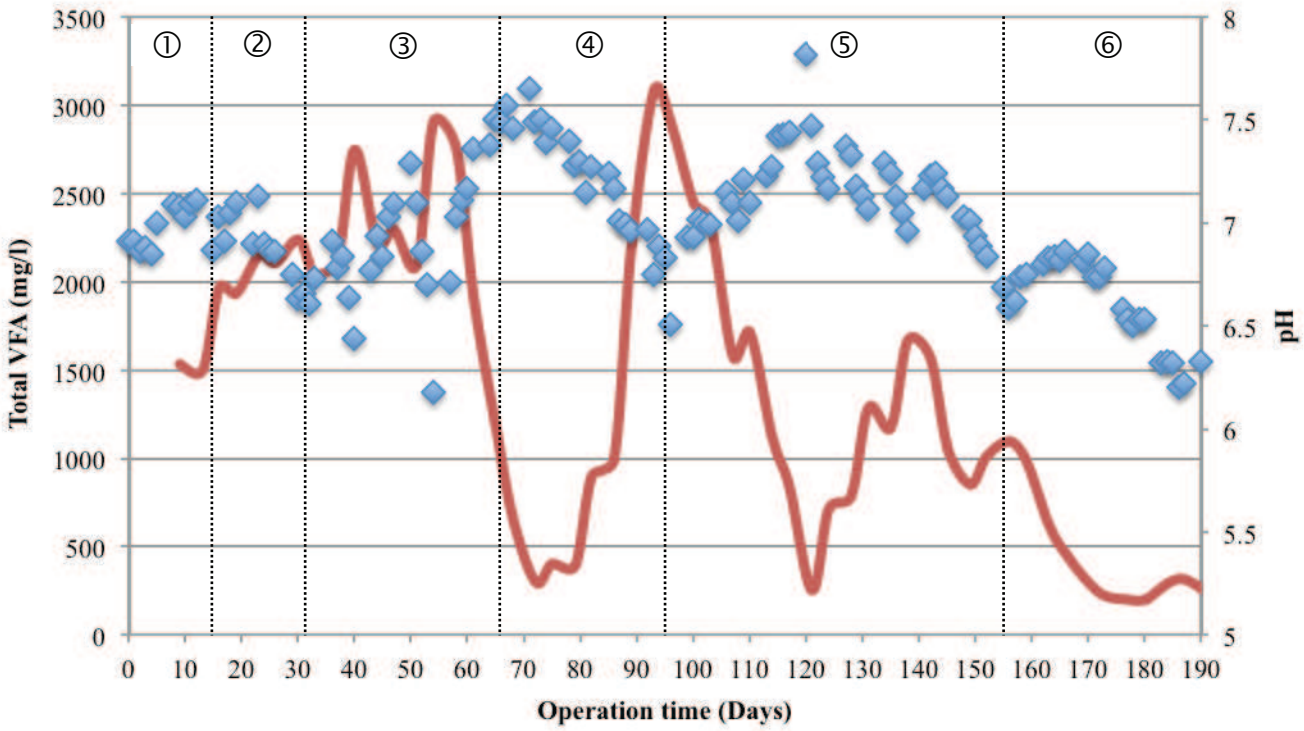

Figure 12.4. Total VFA (—) and pH (◆) over 190 days.

Feeding was resumed on Day 36, stopped on Day 40 and resumed again on Day 50, and this led to further increases in VFA concentrations. Thereafter, feeding was stopped and the digester was left to recover until Day 66. A gradual raise in the OLR associated with alternating feeding resulted in an increase in biogas production but VFA levels again reached concentrations up to 3000 mg/l, which impacted directly on pH. The two remaining stages of the experiment were conducted with green peas only but each increase in the loading rate brought about an increase in VFA concentrations. pH values during the last stage of the experiment (⑥) were below 7 and the alkalinity of the system was found to be decreasing from Day 125 (Figure 12.5).

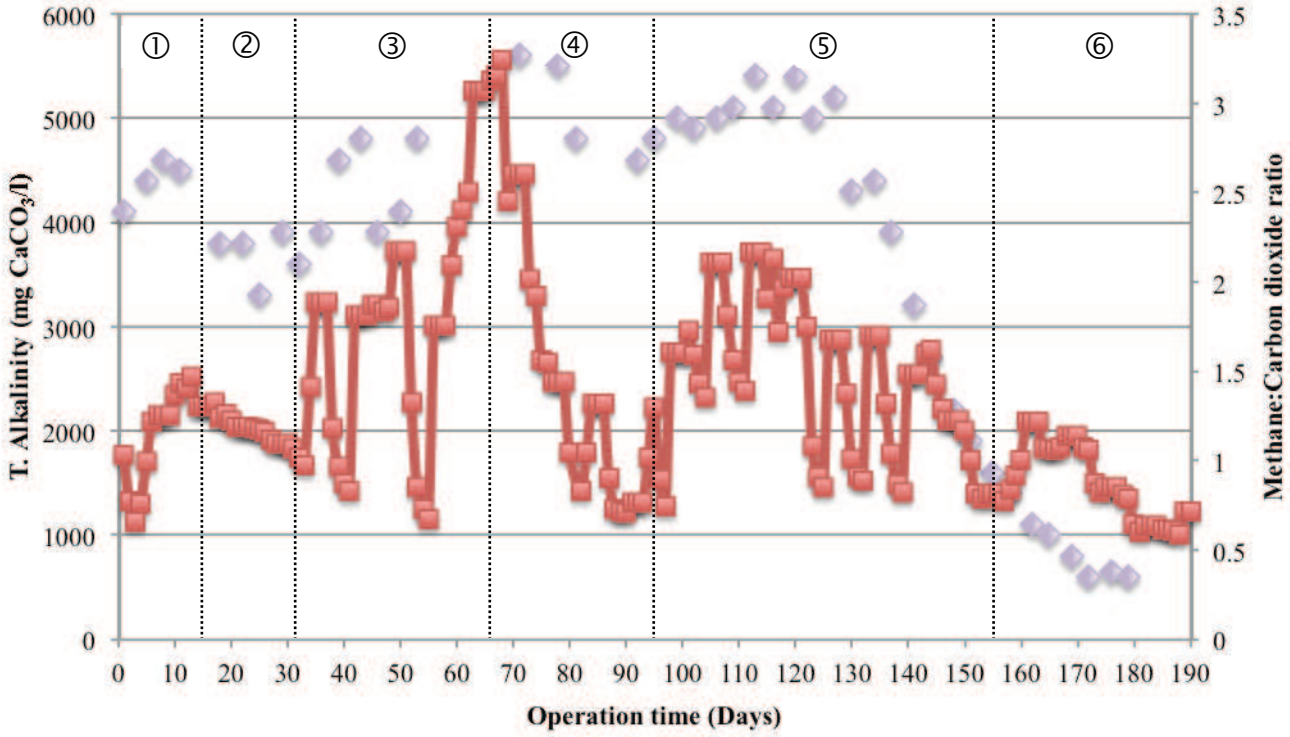

Figure 12.5. Total alkalinity (◆) and CH_4:CO_2 ratio (■) over 190 days.

The methane to carbon dioxide ratio was also found to vary extensively over the experiment, which was an indication of process imbalance. Salt and hydrogen sulphide (H_2S) levels were found to be below inhibitory concentrations. Similarly, ammonium levels were measured throughout the assay and the resulting concentration of ammonia was not believed to be detrimental as shown on Figure 12.6. Organic matter removal efficiency was not calculated since the system did not reach steady state. Effluent COD levels were found to be at similar range during most of the study, but decreased significantly after Day 106, presumably due to the lower quantity of organic matter introduced in the system. Results also show that the addition of a small fraction of brown seaweed to a digester treating vegetable residues at high OLR can bring about a strong imbalance in microbial activities, to the detriment of methanogenesis.

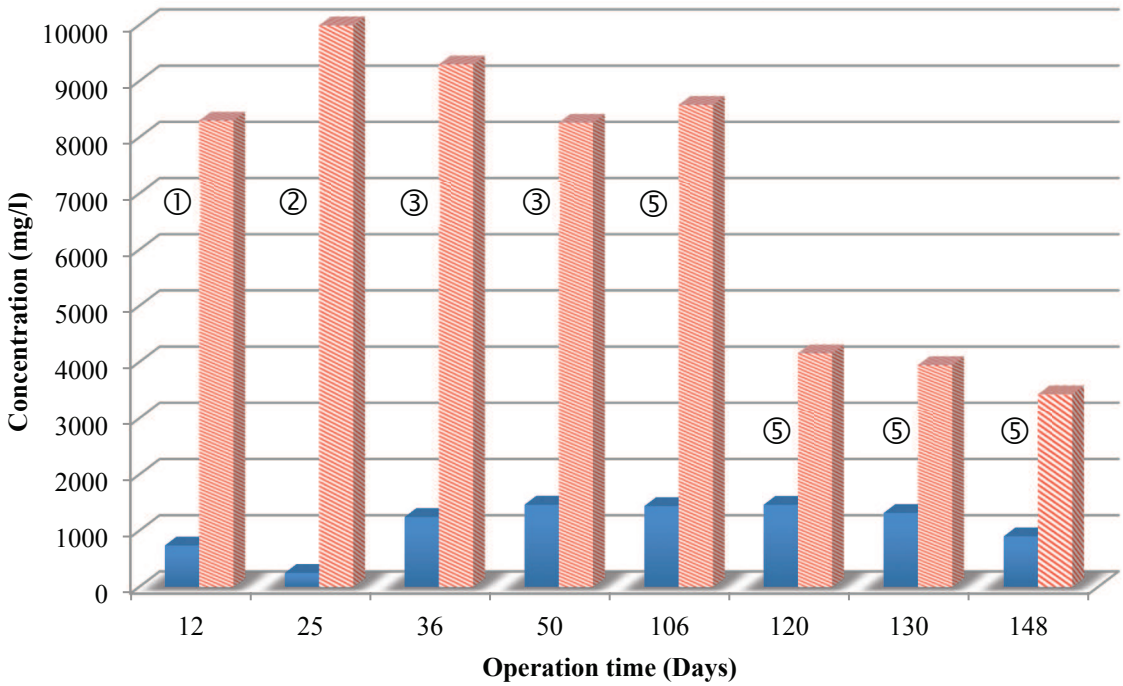

Figure 12.6. Ammonium (solid blue bar) and soluble COD (diagonal red bar) concentrations between Day 12 and Day 148.

In the light of all parameters assessed, it can be assumed that certain compounds contained in the seaweed may be proportionately more inhibitory to methanogenic anaerobes than to other microbial groups in the system especially at relatively high organic loading rates. Furthermore, the limited buffering capacity of the system could not prevent the decrease of pH below neutral values, which also impacted negatively on methanogenesis. When studying the anaerobic digestion of a mixture of fruit and vegetable waste, Mata-Alvarez *et al.* (1992) found that the maximum OLR that could be achieved during the anaerobic conversion of vegetables residues was generally below 3 kg $VS.m^{-3}.day^{-1}$, close to the high loading rate used at the beginning of this assay. Consequently, the experiment was repeated at a reduced loading rate.

12.3.2. System performances at reduced loading rate

Total biogas and methane productions are shown on Figure 12.7. VFA concentrations and pH can be seen on Figure 12.8. From Day 11 to 42, biogas production increased from 1.6 l/day to about 2 l/day, whilst methane yields remained stable when seaweed represented 2% to 5% of the total organic mass input. At Day 43, seaweed was increased to represent 10% of the total OLR (0.77 kg $VS.m^{-3}.day^{-1}$) and both biogas and methane production dropped substantially and resulted in the increase of total VFA levels.

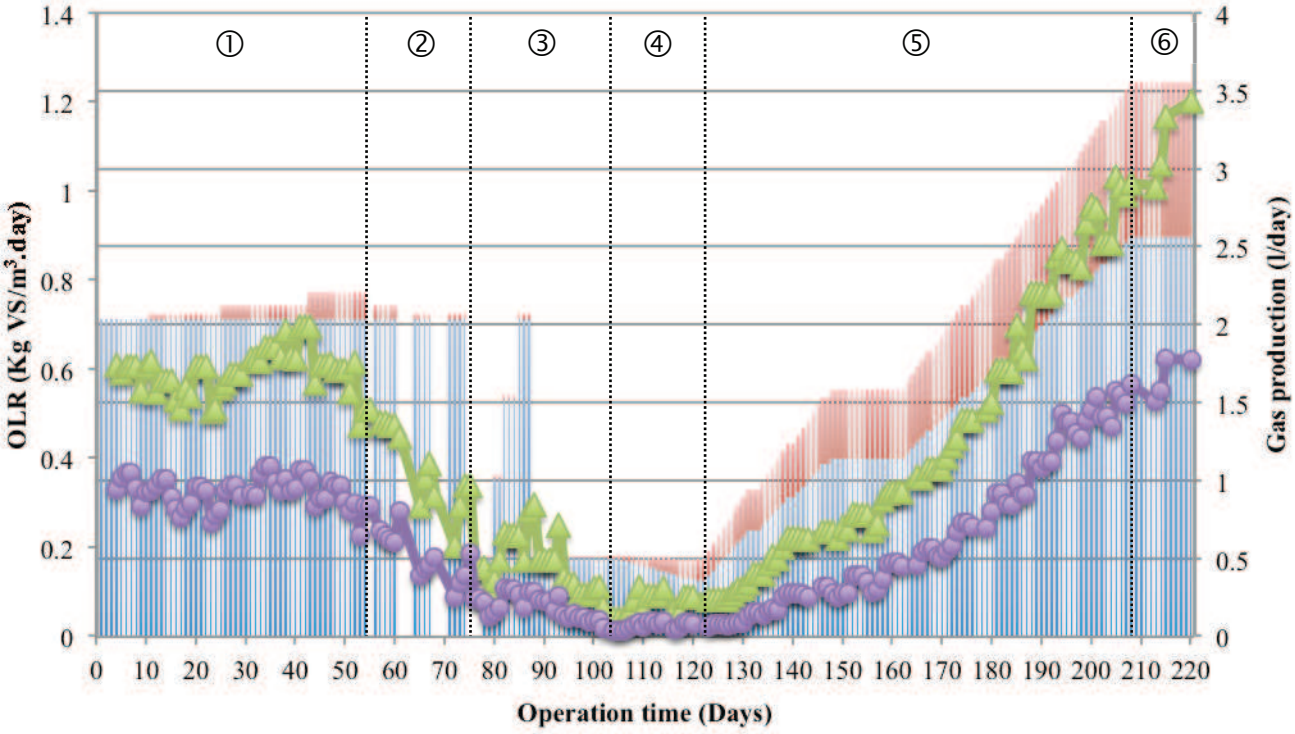

Figure 12.7. Biogas production: ▲ total; ● methane and OLR: ■ vegetable residues; ■ seaweed over 220 days.

In an attempt to limit the accumulation of fatty acids and maintain pH near to neutral values, the system was operated in a sequence alternating feeding at varying loading rates and periods without feeding between days 54 and 75.

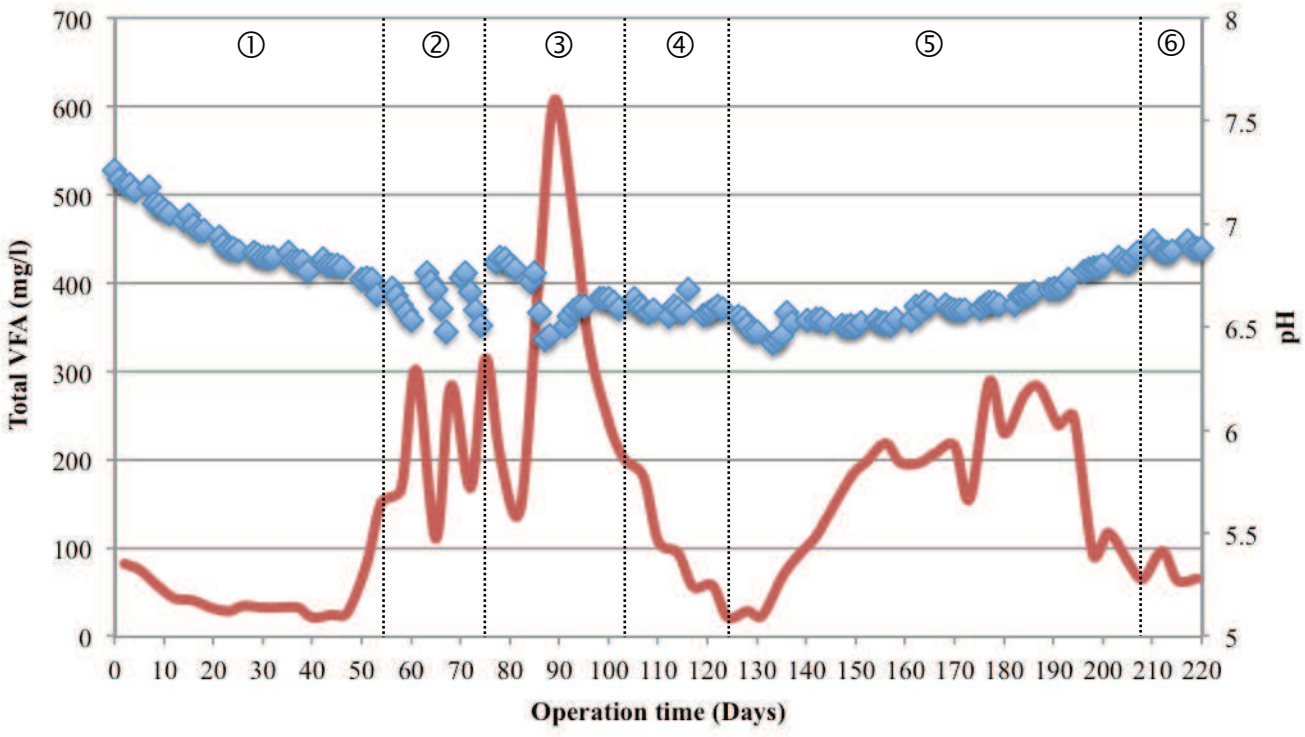

Figure 12.8. Total VFA (—) and pH (◆) over 220 days.

During this period, VFA concentrations and pH levels fluctuated rapidly. At Day 75, $CaCO_3$ was added daily in order to increase the system buffering capacity. Feeding was started again on Day 78, but a sharp increase in the OLR resulted in the accumulation of VFA and a decrease in the production of methane. Feeding was then conducted at a loading rate of 0.18 kg $VS.m^{-3}.day^{-1}$ between Day 90 and Day 122 and until the concentration of fatty acids decreased considerably. During this period, the loading rate was kept constant whilst the percentage of seaweed was increased, resulting in a slight augmentation in biogas production and stable pH levels. On Day 122, the gradual raise of the loading rate for a period of 106 days resulted in the increase of both biogas and methane production to 3.4 l/day and 1.8 l/day respectively with stable pH values and relatively low VFA levels. Figure 12.9 shows the total

alkalinity and the methane to carbon dioxide ratio. Total alkalinity levels were found to continuously decrease during the first phase of the experiment.

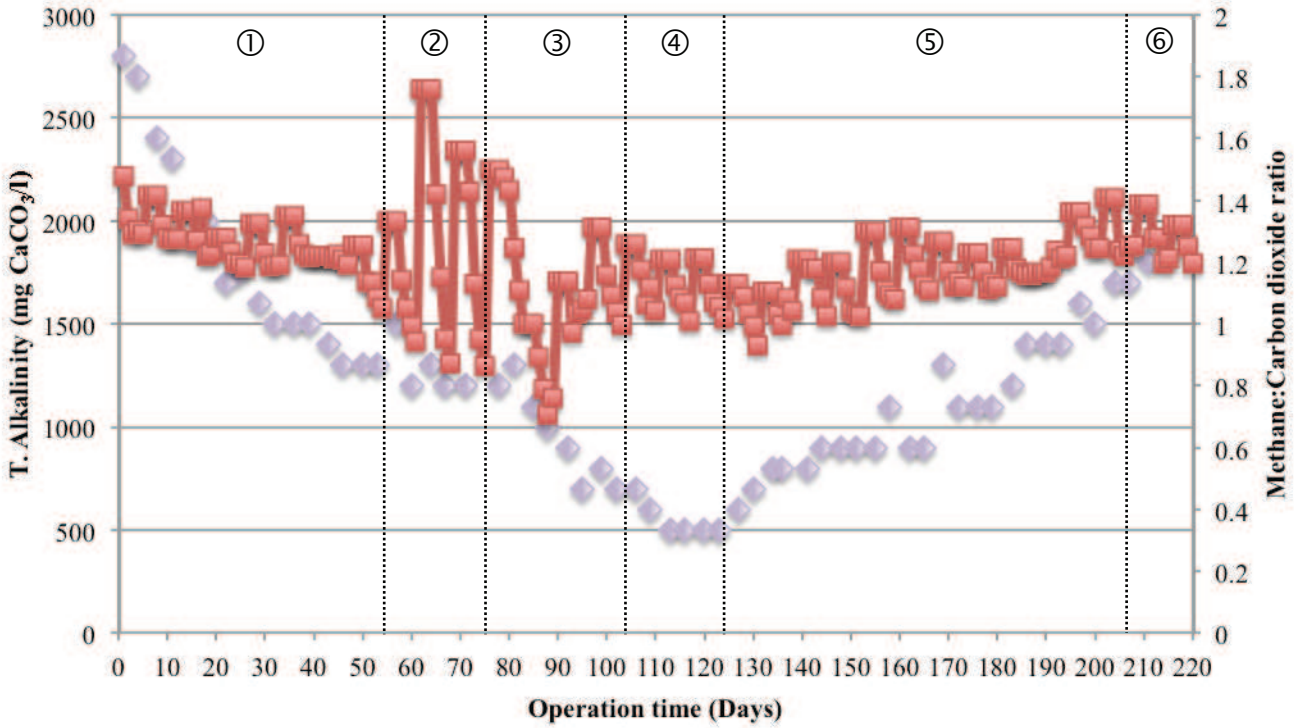

Figure 12.9. Total alkalinity (◆) and CH_4:CO_2 ratio (■) over 220 days.

The addition of calcium carbonate on Day 75 resulted in a slight increase of the system alkalinity, but levels rapidly decreased on Day 88 corresponding with an increase in VFA concentrations. During the last two stages of the experiment, alkalinity levels increased steadily. The CH_4:CO_2 ratio was found to decrease during periods of instability (② and ③). From Day 102, the ratio increased again and was relatively stable during stages in which the system was fed without interruption (④,⑤ and ⑥). Salts levels were kept below inhibitory concentrations and there was no significant increase in H_2S production. Similarly ammonium levels were measured regularly and levels were not considered to be detrimental (Figure 12.10). Effluent

COD increased after Day 120 presumably due to the higher quantity of organic matter introduced in the system.

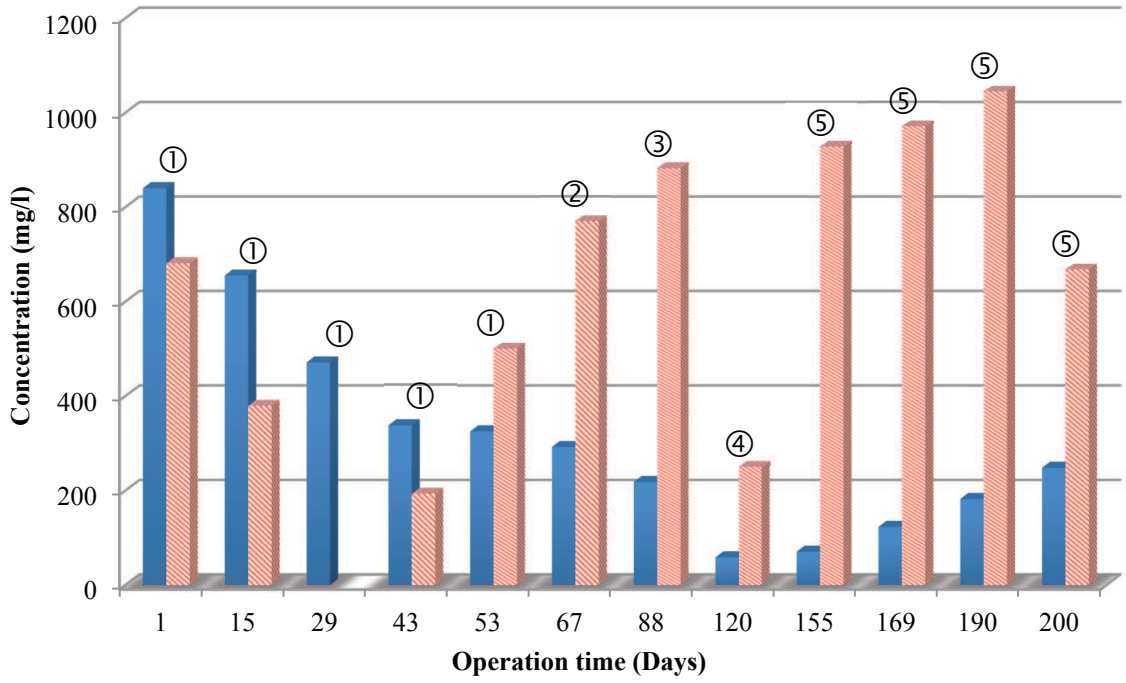

Figure 12.10. Ammonium (solid blue bar) and COD_S (diagonal red bar) concentrations between Day 1 and Day 200.

Results show that relatively high proportions of *Laminaria digitata* in the feedstock can bring about VFA accumulation and system instability, which may be difficult to reinstate. This instability does not seem to be resulting from high levels of known inhibitors such as H_2S, NH_3 or salt levels, since these compound were found to be below reported inhibitory thresholds. The addition of calcium carbonate helped to maintain stable pH values and alkalinity levels, which remained between 1500 and 2000 mg $CaCO_3$/l. During this period it was possible to progressively raise both the total organic loading rate and seaweed content for the latter to represent up to 39% of the total mass input. This resulted in high methane production and stable values for intermediary indicators. Figure 12.11 shows a comparison of the specific gas

productions (calculated using Equation 6.11) for both high and low rates experiments. The figure shows similar results at the beginning of both studies when only green peas were used. At high loading rate, the specific gas production varied during the whole experiment, whilst during the second experiment it was found to increase steadily from Day 130 and reach values between 0.4 and 0.5 m^3/kg VS_{added}.

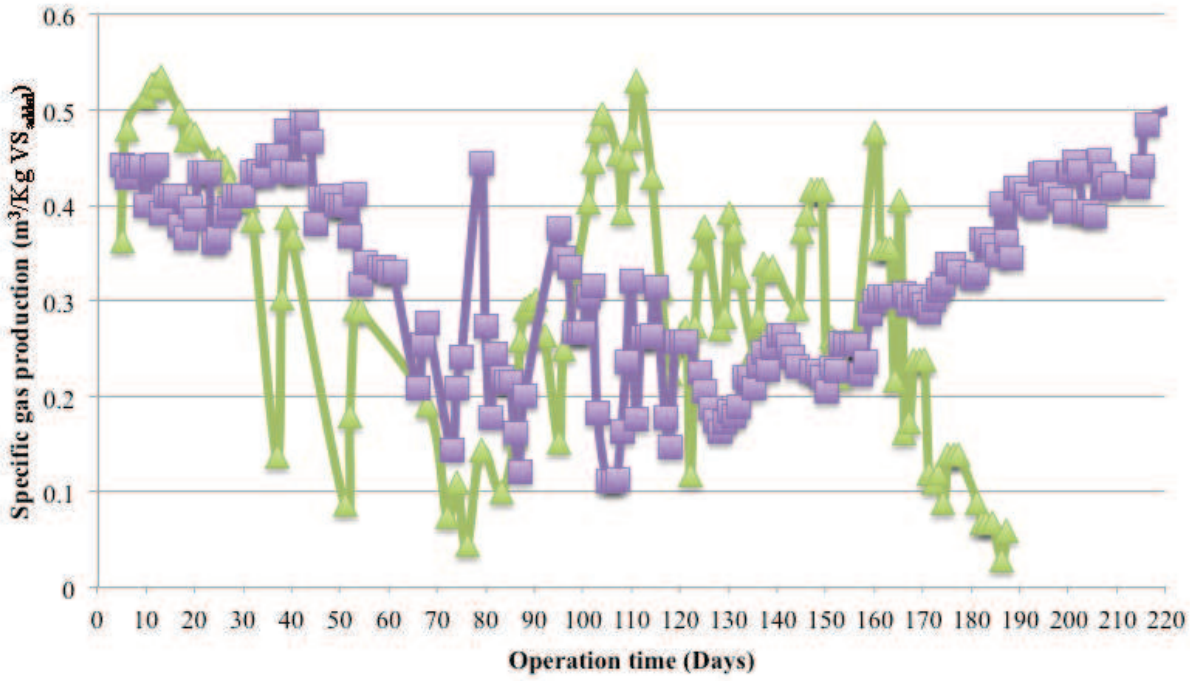

Figure 12.11. Specific gas production: ▲ high loading rate (190 days); ■ low loading rate (220 days).

In both experiments, it was observed that the addition of brown seaweed resulted in a rapid build up of VFA concentrations, which indicates that methanogens are more adversely affected by some constituents of the seaweed. Based on findings reported in Chapter 10, it is likely that phlorotannins are the major cause of the observed inhibition with their detrimental effect further increased by a hydraulic retention time that did not support biomass adaptation and might have resulted in microbial washout due to the slow growth rate of methanogens.

12.4. Conclusions

In this study, it was found that the poor system performances observed during the co-digestion of vegetable residues and brown seaweed could not be explained by the accumulation of common inhibitors such as hydrogen sulphide, salt or ammonia, whose measured levels remained low throughout both experiments. The most probable explanation is the antimicrobial effect of phlorotannins on methanogens, which might have been further intensified by the operational parameters used in these assays. Although, the use of seaweed as an alternative organic substrate in anaerobic digestion systems has been found possible, its phlorotannin levels might prevent commercial-scale applications.

Chapter 13
Conclusions and future perspectives

This final chapter presents a summary and discussion of the experimental results obtained. It also contains a general conclusion and suggestions for future work.

13.1. Summary of results and discussion

This work was conducted in order to investigate the potential of brown seaweed to serve as an additional source of organic matter for anaerobic digesters. The research focused on the effect of salinity and phenolic compounds on process performance. The anaerobic co-digestion of brown seaweed and vegetable residues was also studied in laboratory-scale anaerobic digesters in order to determine the optimum operational conditions. The Anaerobic Digestion Model No.1 (ADM1) was modified to include the inhibition processes observed from laboratory-conducted experiments. Experimental studies were carried out in three main stages. The first stage investigated the effect of salinity on acetoclastic methanogens in both batch and reactor systems. The adaptation of microorganisms to salinity was also studied and modelled. In the second stage, the effect and mode of action of phenolic compounds contained in brown seaweed was studied qualitatively using transmission electron microscopy and quantitatively by the measurement of microbial intra-cellular material leakage through spectrophotometric methods. The third stage investigated the factors

affecting co-digestion of brown seaweed with vegetable residues. The paragraphs below summarise results obtained for both process modelling and experimental stages and discuss findings in the context of the current literature.

Parametric sensitivity analysis techniques were used on the ADM1 parameters representing the model initial conditions, which are of utmost importance on model predictions. It was found that parameters representing the initial concentrations of inorganic carbon, inorganic nitrogen and cations have the highest impact on the predicted biogas flow. Results also showed that the variation of these parameter initial values impacted upon the model output of interest for a period corresponding to two complete hydraulic retention times. After this period, the model predictions were determined mainly by the influent feedstock characteristics. The estimation of these parameters' initial conditions, together with the complete feedstock characterisation, are likely to provide more accurate results for the modelling of sodium and phenolic compounds inhibition. A similar analysis conducted for input parameters could reduce the difficulties associated with the systematic characterisation of the substrate chemical composition. The use of local sensitivity analysis methods applied to the ADM1 parameters has been reported in the literature (Batstone *et al.* 2002; Tartakovsky *et al.* 2008; Lee *et al.* 2009; Silva *et al.* 2009), but this is the first time that results from global sensitivity analysis techniques are presented. Global sensitivity analysis, which takes into consideration the interactions between parameters or group of parameters over the output, is likely to provide more meaningful results than local sensitivity analysis when used for non-linear and highly dynamic models. This work also considers for the first time the use of such tools on the ADM1 initial conditions and suggests that the effect of the latter tends to decrease over time.

In the first experimental stage, sodium bicarbonate was added at regular intervals to an anaerobic reactor treating vegetable residues in order to assess the effect of sodium ions on acetoclastic methanogens. Results showed that each addition of sodium negatively impacted on biogas production through the inhibition of methanogenesis and as evidenced by the rapid accumulation of volatile fatty acids. The ADM1 structure was modified to include a non-competitive function representing the effect of sodium ion concentration on the rate of acetate uptake. The calculation of sodium concentrations within the system was also implemented. The model was found to accurately predict biogas production, pH values and total volatile fatty acids concentrations. The modified ADM1 was used to project the effects of varying concentrations of sodium ions in the presence of other known process inhibitors. An anaerobic digester characterised by fluctuating levels of ammonia nitrogen and sodium was considered and it was found that low sodium ion levels can result in process inhibition in the presence of high levels of ammonia. However, the tolerance threshold for sodium ions increases when the concentration of ammonia is relatively low. These results can be generalised to anaerobic digesters, in which considerable amounts of sodium hydroxide are used for pH control and during the anaerobic digestion of proteinaceous substrates. The inhibitory effect of sodium ions on anaerobic digestion processes is well documented (Feijoo *et al.* 1995; Chen *et al.* 2008; Iacovidou *et al.* 2012; Suwannoppadol *et al.* 2012) with results obtained mostly from batch tests. This work presented studies of reactor systems, which is novel. Sodium concentrations varying between 0.24 to 2.3 mol/l have been shown to decrease methanogenic activity by about 50% (Soto *et al.* 1993; Feijoo *et al.* 1995; Chen *et al.* 2008; Jeison *et al.* 2008) and approximate the values determined in this study of 0.21 mol/l, thus in accordance with inhibitory levels reported in the literature.

The ADM1 has seen the addition of numerous inhibition processes, but this work is the first to implement an inhibition function to represent the effect of sodium ions on acetoclastic methanogens. Microbial adaptation to salinity was also investigated in batch tests using brown seaweed as a saline-rich feedstock. Results showed that non-saline adapted cultures produced relatively small amounts of methane during the first 15 days of the assay. This period was believed to correspond to an adaptation phase during which the microorganisms were adapting to the increased salt concentration. The ADM1 was modified to include the effect of salinity on acetoclastic methanogens and the value of the maximum uptake rate for acetate was adjusted to represent the lag phase observed for methane production. High correlation values found between observed and predicted values of methane production indicated the validity of the modelling approach and parameters estimation. From the results obtained, it was postulated that an adequate adaptation period can significantly reduce the adverse effects of salinity. It was concluded that the use of saline-tolerant cultures is more appropriate in systems consistently treating saline-rich feedstocks, because of their salt requirement, whilst non-saline cultures can be successfully used at varying levels of salinity on the condition that a sufficient period of adaptation is provided. The adaptation of anaerobic microorganisms to inhibitory levels of long-chain fatty acids (Palatsi *et al.* 2010), acetate (Lins *et al.* 2012) or sodium (Soto *et al.* 1991; Feijoo *et al.* 1995; Omil *et al.* 1995; Lefebvre *et al.* 2006) through the use of alternative sources of inoculum or stepwise increases of toxicant concentrations has been extensively covered in the seminal literature, but this work compares for the first time the performances of salt-tolerant and non-saline sources of inocula and the adaptation period necessary for both cultures to reach similar methane yields. Additionally, the implementation of sodium in the ADM1 structure as proposed by Hierholtzer and

Akunna (2012) has been extended to include the concentrations of other cations such as calcium, magnesium and potassium, and kinetic parameters were adjusted in order to model the lag phase observed for methane production. Batstone *et al* (2003) used a similar strategy to represent a delayed uptake of valerate during anaerobic oxidation at thermophilic temperatures, but the successful modelling of microbial adaptation to salinity is reported for the first time in this work.

In the second experimental stage, the effect and mode of action of phenolic compounds on mixed microbial cultures was investigated. Phlorotannins were extracted from *Laminaria digitata* and added at different concentrations in batch containing anaerobically digested sludge as a source of inoculum. Results showed that final methane yields were inversely proportional to phlorotannin concentration, whilst results of leakage assays suggest that the compound can adversely affect cell membrane permeability. Results also indicated that the bactericidal activity of phlorotannin is a function of the degree of polymerisation of the compound and are in accordance with findings from Nagayama *et al.* (2002). However, this work is the first study to investigate the inhibitory effect of phlorotannin on mixed anaerobic microbial cultures and impact on anaerobic digestion systems with current research focusing on the effect of phlorotannin on bacteria such as *Escherichia coli* (Wang *et al.* 2009), *Campylobacter jejuni* (Nagayama *et al.* 2002) or *Staphylococcus aureus* (Eom *et al.* 2008), since the compound is believed to have potential applications as an antimicrobial agent for the pharmaceutical and food industries (Li *et al.* 2011; Thomas and Kim 2011; Eom *et al.* 2012). This work also posits a probable mode of action for the antimicrobial effect of phlorotannins on anaerobic microorganisms, which could jeopardise the use of brown seaweed as a suitable feedstock for anaerobic digestion. In addition, the co-digestion of brown seaweed and vegetable

residues was investigated at mesophilic temperatures. It was found that the addition of seaweed to represent a weight ratio of 10% of the total organic loading rate resulted in strong system imbalance in disfavour of methanogenesis. In the light of all parameters measured, it was concluded that the system imbalance resulted from the inhibitory effect of phlorotannin on methanogens. The ADM1 was modified to include the characteristics of both substrates and calculate the resulting inflow. An uncompetitive function was added in the model to consider the inhibitory effect of phlorotannin on acetoclastic methanogens. In order to represent the adaptation of microorganisms, an alternative microbial population with an increased tolerance to phenolic compounds was further added in the model original structure. The modified model was able to accurately represent the system imbalance corresponding to the introduction of seaweed in the reactor. It was concluded that the calibrated model could be used to predict changes induced by phlorotannin on methanogens populations. The co-digestion of brown seaweed and vegetable residues is presented and successfully modelled for the first time in this study. The biodegradation of phenolic compounds has been previously implemented in the ADM1 by Fezzani and Ben Cheikh (2009), but this study further considers the development of an alternative population of acetic acid degraders characterised by a different level of tolerance to phlorotannin.

The third stage of this work investigated the co-digestion of brown seaweed with vegetable residues at different organic loading rates and proportions of substrates in an anaerobic digester operated at mesophilic temperatures. Results showed that the addition of seaweed impacted negatively on methane yields and volatile fatty acids levels immediately increased. Attempts to stabilise the process through reduced loading rates were not found effective and it was concluded that the system might have been impacted by the inhibitory effect of phlorotannin. However, the increase of

the buffering capacity through the addition of calcium carbonate was efficient to maintain pH near to neutral values and by gradually increasing both the proportion of seaweed in the influent and the total loading rate, the digester could be successfully operated. There has been very little literature on the anaerobic co-digestion of brown seaweed and this is the first study to establish operational guidelines for the co-digestion of brown seaweed with non-saline feedstock. Matsui and Koike (2010) investigated the feasibility of co-digesting macroalgae with milk, but used a mixture of brown and green seaweeds in a two-phase reactor configuration with pH control. Gurung *et al.* (2012) recently evaluated the use of brown seaweed as a potential feedstock for anaerobic digestion and concluded that co-digestion of marine biomass with nutrient-deficient wastes can enhance methane yields obtained from these substrates. However, the authors did not report any specific co-digestion results and conducted their experiments through batch tests in which operational conditions are significantly different from continuously and semi-continuously fed systems. Lee *et al.* (2012) studied the biogas production of the brown seaweed *Laminaria japonica* and focused on the use of specific microorganisms cultures, yet the relevance of these results in a full-scale anaerobic digester was not reported. Overall, this study has shown that brown seaweed can be considered as a potential source of organic matter for anaerobic digesters but inherent phenolic compounds could limit their commercial-scale application.

13.2. General conclusion

The effects of salinity and phlorotannin on methanogenesis have been shown to be the main factors influencing the successful anaerobic digestion of brown seaweed. This study established that the impact of sodium ions on acetoclastic methanogens could be

mitigated by the adaptation of microorganisms to increasing levels of salinity. The effect of phlorotannin has been suggested to be a function of the polymerisation level of the compounds and has been found to induce strong extra- and intra-cellular effects on microorganisms. Results also showed that anaerobic digestion can be effective at relatively high concentrations of phlorotannin providing sufficient time is given for the development of phlorotannin-tolerant microorganisms. The use of the Anaerobic Digestion Model No.1 has been found effective for the control, operation and optimisation of anaerobic systems through the addition of specific inhibition mechanisms. The use of systematic sensitivity analysis methods has also been useful to determine the model inputs requiring a careful determination. In general, it can be concluded that the effective use of brown seaweed as an additional source of organic matter for anaerobic digestion is dependent on the concentrations of two main inhibitors naturally found with the substrate, *viz*., salinity and phlorotannin. Seasonal variations in phlorotannin levels are likely to further increase the complexity of brown seaweed as a suitable substrate. Microbial adaptation is the key to guarantee process stability and efficient conversion rates in the digestion of brown seaweed species and this can be enhanced by the appropriate operation of anaerobic systems. Co-digestion can therefore be considered as an appropriate method to contribute to the overall reduction of inhibition by means of dilution and a greater flexibility for system operation. This work has shown that modelling tools play an increasingly important role to the understanding and prediction of anaerobic digestion. The capability to forecast system performances when subjected to varying substrate conditions is crucial in increasing the economic value of biogas production from anaerobic digestion systems.

13.3. Future perspectives

The main research direction that has emerged following this work relates to the effect of phlorotannins on microorganisms and methods to mitigate their impact. The neutralisation of phlorotannins by fixation with formaldehyde at concentrations that would prevent the formation of an insoluble phenol-formaldehyde resin as observed by Moen *et al.* (1997) is of particular interest. In order to optimise the use of chemical fixatives, such studies should be preceded by the exact chemical determination of phlorotannin compounds and their degree of polymerisation. Alternatively, phenolic compounds could be separated in solutions by adsorption-desorption (Soto *et al.* 2011). The identification of microorganisms naturally tolerant to phlorotannin and ways of microbial adaption to phenolic compounds should also be investigated. The transformation of brown seaweed to alternative biofuels such as ethanol has been studied (Adams *et al.* 2011b) but little has been done to assess the most suitable bioconversion process in terms of net energy yields. As observed in this work, acid-producing microorganisms seemed less affected by inhibitory compounds when compared to methanogens and recent research has focused on the relatively high production of volatile fatty acids from seaweed under anaerobic conditions and the conversion of these acids to a mixed alcohol fuel (Pham *et al.* 2012). However, the relative amount of energy produced through the latter method when compared to anaerobic digestion remains unclear and there is need for further investigation. The effectiveness of other process configurations such as two-phase anaerobic systems contributing to the development of optimised conditions for hydrolysis/acidification in a first stage and methanogenesis in a second stage should also be considered and might improve the adaptation of the more sensitive methanogens.

The modified version of the ADM1 could be further improved to consider the seasonal variation of the substrates considered in this study and hence be applied to determine the most suitable substrate ratio depending on the availability, seasonal composition and concentration of inhibitory compounds characterising the feedstocks. With the implementation of alternative microbial species, the adaptation level of different microorganisms to both salinity and phlorotannins could also be taken into account and therefore represent an alternative to heuristic approaches, which could lead to serious consequences on anaerobic systems and induce strong imbalances between the rates of the different steps involved in the overall anaerobic biodegradation (Zaher *et al.* 2009b). The modelling of anaerobic digestion has also been limited in its use because of the lack of suitable benchmarks and the unavailability of databases that render the reproducibility of published results laborious (Donoso-Bravo *et al*. 2011). A structured and freely available collection of data relating to the use of the ADM1 for different substrates and system configurations might be beneficial to this regard. The use of systematic mathematical tools and clearly defined procedures to ensure both the validity of the model modifications and the accuracy of parameters might also promote the acceptance of modelling tools to plant operators and engineers.

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